Scientific Edition

JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXV — *MAY, 1946* — NUMBER 5
CONSECUTIVE NO. 10

Diffusion of Sulfonamides from Emulsified Ointment Bases*

By C. LEE HUYCK, RUBY S. HIROSE†, and PEDRO A. REYES, JR.‡

Twenty-four of the investigators reviewed have compared the results of clinical investigation of sulfonamides suspended in oil-in-water base with sulfonamides suspended in water-in-oil base, grease base, or water-miscible base. *In vitro* tests indicate that the sulfa drugs suspended in an oil-in-water emulsion type ointment base diffuse more readily than sulfa drugs suspended in the water-in-oil emulsion type ointment base. *In vitro* tests indicate that diffusion increases with the miscibility of the base. The addition of a large amount of the wetting agent dioctyl sodium sulfosuccinate (Aerosol OT) increases the diffusion of sulfa drugs suspended in the water-in-oil emulsion type base. The addition of lanolin to triethanolamine emulsions containing sulfathiazole increases slightly the diffusion of sulfathiazole.

REVIEW papers (1–4) reveal that there is some difference of opinion as to whether a water-in-oil emulsion type ointment base or an oil-in-water emulsion type ointment base is better for the topical application of sulfa drugs. The true answer to this question can probably be obtained only by extended clinical study. However, some indication of the comparative efficiencies of these two types of ointments can be gained by measuring the diffusion of sulfonamides from the two types of ointment bases over a period of time. The experimental work reported in this paper is comparative measurement of the diffusion of sulfa drugs from the two types of ointments over specified periods of time.

As an emulsified ointment base for sulfonamides Mumford (5) obtained excellent results with three parts of liquid paraffin, two parts of white petrolatum, and two parts of a mixture of higher aliphatic alcohols (hexadecyl and octadecyl) containing 10 per cent phosphated acid esters of the alcohols. The base was neutral and stable in the presence of acids, alkalies, metals, and salts. It was emulsified in the presence of water and was easily washed off the skin and fabrics.

Robson and Wallace (6) used a glycerin-

* Received Sept. 21, 1945, from the Research Laboratories, The Wm. S. Merrell Co., Cincinnati, Ohio.
† Kettering Laboratories, University of Cincinnati.
‡ Inter-American Trade Scholar; present address: av. J. Bertis 14, Villa Delgado, El Salvador, Central America.

sulfanilamide paste in the treatment of burns. The paste was used with success in second and third degree burns of the hands, face, scalp, and buttocks. This paste did not possess the disadvantages associated with the tannic acid and saline methods of treatment. In third degree burns the paste allowed an early separation of slough, control of sepsis, and development of a healthy granulating surface for early skin grafting. In small second degree burns the paste caused slight pain on application in a few cases but later on it was soothing, nonirritating, and healing.

In an answer to a query made by Miller (7) as to what would be a good base for sulfanilamide ointment, the editor of "Queries and Minor Notes" Department of the *Journal of the American Medical Association* recommended an ointment base containing sodium alginate, water, anhydrous wool fat, and petrolatum. He states that it could not only be applied to surface wounds but also to mucous membrane surfaces such as the conjunctiva.

Robinson and Robinson (8) tested clinically sulfathiazole ointments of vanishing cream and grease bases and concluded that sulfathiazole ointment of either grease or greaseless base was effective in the treatment of coccogenous dermatosis.

Keeny, Pembroke, Chatard, and Ziegler (9) used a 5 per cent sulfathiazole ointment with a vanishing cream base containing hydrous wool fat on 69 patients with various cutaneous infections and reported that it appeared to be efficacious in the treatment of infected infantile and adult eczemas, seborrheic dermatitis, impetigo, acne vulgaris, bacterial folliculitis and furunculosis. Thirty-five patients with acute vesicular poison ivy dermatitis, 33 patients with cuts and four patients with small second degree burns received local applications of the ointment. Lesions of all patients healed without infection.

Later Keeney (10) reported that 5 per cent sulfathiazole in a base of 25 per cent Aquafor and 70 per cent cold cream gave results which were superior to the results obtained with the vanishing cream base previously tested.

Veal and Klepser (11) used 10 per cent sulfanilamide, 2 per cent allantoin, and 0.5 per cent chlorobutanol in a triethanolamine glycerin stearic acid washable ointment base on infected wounds, burns, leg ulcers, decubitus ulcers, and traumatic wounds. In practically all the infected cases treated, there was a mixture of organisms present, the most common invaders being streptococcus, staphylococcus, the colon group, and diphtheroids. In some cases the growth of *Bacillus pyocyaneous* was extensive. Regardless of the bacteria found, this ointment caused an immediate inhibition in the growth of the various organisms.

Pillsbury, Vammock, Livingood, and Nichols (12) found sulfathiazole ointment with an oil-in-water emulsion base to be effective in the treatment of primary superficial infections of the skin, including staphylococcic or streptococcic impetigo, eczematoid dermatitis, Bockhardt's follicular impetigo and superficial ecthyma. It was equally effective in acute secondary infections of various pre-existent dermatoses, including particularly pyogenic complications of fungus infections and dermatitis venenata. Certain criteria for the effective local application of sulfathiazole were established and an oil-in-water emulsion vehicle in which the sulfathiazole was suspended in the external aqueous phase apparently met these requirements satisfactorily. A vanishing cream type stearate base was somewhat less effective than the emulsion type base while the grease base proved definitely inefficient. The sulfathiazole ointment was useful in chronic deep ulcers of the skin and subcutaneous tissues. In various chronic eczematoid lesions, from which large numbers of staphylococci or streptococci might be recovered, the effects of sulfathiazole ointment were much less striking than in impetigo but still this treatment was occasionally very helpful. There was no evidence of absorption of sulfathiazole in the patients treated and no local or general toxic reactions occurred. Superficial infections caused by streptococci responded somewhat more rapidly and more completely than those caused by staphylococci.

Guyton (13) used an ointment containing

sulfanilamide, sodium alginate, sodium chloride, Aquafor absorption base, and vaseline. This ointment proved to be the most effective of the preparations the author had tried in obtaining a high aqueous concentration of sulfanilamide in the conjunctival sac without too frequent instillations. The reasons advanced for this result were: (*a*) The sulfanilamide in this ointment was in the colloidal form, (*b*) the ointment was only very slightly irritating and therefore was not washed from the eye by excessive lacrimation.

Thiessen and Steinreich (14) treated nine burned patients with a 10 per cent sulfathiazole ointment containing 10 per cent cod-liver oil in a lanolin petrolatum base. All the burns treated were first and second degree but varied in extent. The average period of hospitalization was only 9.4 days which was a shorter period than the periods of hospitalization treated with gentian violet and tannic acid. In addition the healed surfaces were smoother than surfaces treated with tannic acid and gentian violet. Despite a large raw surface, there was either no absorption of sulfathiazole or the absorption was too small to be measured by blood level determination.

Glicklich (15) used sulfathiazole in equal parts of lanolin and boric acid ointment on 42 patients with dermatologic diseases caused by pyogenic organisms. The strength of the ointment made little difference and infections cleared more quickly than infections treated by other means. The application of sulfathiazole to the skin for forty-eight hours caused the appearance of only an insignificant amount of drug in the blood stream.

Miller (16) used the sulfonamides in strengths varying from 5 to 50 per cent in an oil-in-water base containing diethanolamine oleate, white wax, liquid petrolatum, white petrolatum, and distilled water. Successful results were obtained in the treatment of impetigo contagiosa, ecthyma, folliculitis, acne varioleformis, and infantile eczema.

Allen, Owens, Evans, and Dragstedt (17) considered three features in the choice of an ointment base: (*a*) The base itself should be nontoxic, (*b*) the base should be absorbent since such a base theoretically tends to minimize the absorption of the drug by the tissues, (*c*) the base should permit intimate contact between sulfathiazole and local tissues. With these features in mind, Aquafor containing 5 per cent oxycholesterol and 95 per cent petrolatum was chosen as the base. This ointment was widely employed at the University of Chicago clinics for nearly a year in the treatment of superficial ulcerated areas, abrasions, infected superficial wounds, and in various infected surgical wounds. The results were gratifying in more than 150 cases in which the ointment was employed. The greatest benefits were observed in cases of infection by staphylococcus, streptococcus, or bacilli coli while the least benefit was observed in wounds infected with common anaerobic organisms.

Kalz and Prinz (18) used 5 per cent to 20 per cent sulfathiazole in the following bases: glycerin, petrolatum, cold cream, Aquafor, zinc paste (20 per cent zinc oxide and talc in equal parts petrolatum and lanolin), zinc paste with 5 per cent oil of cade. Glycerin base was the best in that it healed impetigo contagiosa in about half the time required of the other bases. Petrolatum was the poorest base but its action was improved by the addition of oxycholesterol. Zinc paste and zinc paste with 5 per cent oil of cade gave results about equal to the petrolatum oxycholesterol base.

Pillsbury, Schaffer, and Nichols (19) tested the rate at which various bases containing 5 per cent sulfathiazole reduced the bacterial flora of the skin using a standard scrubbing technique. Yellow petrolatum, lanolin, and an oil-in-water emulsion type base were the bases tested. The emulsion base was found to be the best in that it definitely increased the rate at which bacteria were removed from the skin. Yellow petrolatum and lanolin did not increase the rate at which bacteria were removed from the skin.

Ackman and Wilson (20) used an ointment containing 5 per cent sulfathiazole in a triethanolamine, distilled water, white beeswax, and liquid petrolatum base. Emulsions of triethanolamine are characterized

by their stability due to particle size and neutrality. This ointment was introduced as an adjunct to gynecological surgery and the clinical results were so encouraging that further clinical trials seemed justified.

Ackman and Wilson (21) continued their clinical studies with the ointment previously described. Good results were obtained in treatment of appendix abscesses, ruptured appendix with generalized peritonitis, subdiaphragmatic abscess, carbuncles, acute and chronic osteomyelitis, hand infections including palmar abscesses, compound fracture with tissue destruction, open amputation, leg ulcer, large and small infected wounds, minor surgery, and burns.

Gurd, Ackman, Gerrie, and Pritchard (22) used the 5 per cent sulfathiazole oil-in-water emulsion described by Ackman and Wilson (20) in the treatment of burns. The preparation proved to be highly successful in preventing and controlling infections.

Strakosch and Olsen (23) found that sodium sulfathiazole was most rapidly effective in a base of the water-in-oil type when compared with the results of oil-in-water and grease types. A cod-liver oil base rated second best due to the fact that it contained a high percentage of cholesterol. It is useless to incorporate in petrolatum a drug which is soluble in water for it is known that the drug in most instances will not be liberated at all from petrolatum or only in amounts so insignificant that it will have no appreciable effect on the skin. Such a drug must be incorporated either in the oil-in-water or water-in-oil emulsion type ointment base or the polysaccharide base. As a rule a water-in-oil emulsion base penetrated the skin more readily than an oil-in-water or pure hydrocarbon base since it carries the drug into the tissues faster than the other types of bases. Oil-soluble drugs will normally be affected in the reverse manner.

In the treatment of pyogenic infections of the skin, Winer and Strakosch (24) used the 5 per cent sulfathiazole ointment previously described by Strakosch and Olsen (23) and found it suitable for the treatment of impetigo contagiosa. This treatment was more agreeable and cleaner than the former treatments used. The time of healing was shorter than the time required in previous treatments and the treatment was apparently free from dangerous sequelae.

Fuller, Hawking, and Partridge (25) studied the effect of various ointment bases on the rate of absorption of sulfanilamide. *In vitro* it was found that absorption was much delayed by the incorporation of sulfanilamide in oil base or in a water-in-oil emulsion base. The absorption of sulfanilamide was not influenced very much by the use of a glycerinated gelatin base or an oil-in-water emulsion base. The delay in absorption from an oily base was less marked when absorption occurred only from the surface of the product and it was more marked when absorption took place from the deeper layers of the product. *In vivo*, absorption occurred rapidly from all types of preparations when the product was spread over the surface of the mucous membrane in a thin film. When the preparation was embedded in the tissue as a mass so that the surface volume ratio was low, absorption of sulfanilamide was much delayed by incorporation in an oily medium and somewhat delayed by incorporation in an oil-in-water emulsion base. Sulfanilamide was rapidly absorbed *in vivo* from glycerinated gelatin base which was in clinical use for burns and from gauze impregnated with sulfanilamide which was suggested for use as a field dressing.

Elvin (26) suggested three ointment bases for sulfathiazole ophthalmic ointments, the first of which was suggested by Guyton (13). The second base was composed of sodium lauryl sulfate, cetyl alcohol, cocoa butter, white petrolatum, and water. The third contained liquid petrolatum, spermaceti, glycostearin, and water. The three ointments were found to be about equal in therapeutic activity.

Hawking (27) tested the following preparations clinically: (*a*) solution of sulfanilamide lactoside sodium formaldehyde sulfoxylate; (*b*) sulfanilamide, calcium citrate, sodium alginate, spermaceti, and water; (*c*) sulfanilamide, beeswax, cetyl alcohol, oleic acid, triethanolamine, and water; (*d*) sulfanilamide, triethanolamine, stearic acid, white beeswax, wool fat, and water; (*e*) sulfanilamide, liquid paraffin, cetyl al-

cohol, stearic acid, triethanolamine, arachis oil-in-water emulsion; (*f*) sulfanilamide, beeswax, cod-liver oil, oleic acid, sodium hydroxide oil-in-water emulsion; (*g*) solution of sulfanilamide in beeswax and cod-liver oil; (*h*) sulfathiazole in saline; (*i*) sulfadiazine in saline. The author found that persistence of sulfanilamide in a wound was prolonged by incorporating sulfanilamide in an oil medium or by using a less soluble sulfonamide such as sulfathiazole or sulfapyridine. With sulfanilamide the prevention of streptococcal septicemia was attained more completely with an oily preparation than with an aqueous preparation. Histologically, however, the presence of oil in the wound caused undesirable tissue reactions. Of the oils tested, cod-liver oil was least desirable, liquid paraffin was moderately desirable while cottonseed oil was most desirable.

Wilcox (28) treated a number of cases of impetigo with an ointment containing sulfanilamide in a soft paraffin and lanolin base. This base proved effective but the scars remaining after the crusts had disappeared required further treatment.

Wardropper (29) reported that ointments containing sulfanilamide in arachis oil cold cream base did not give very good results in the treatment of impetigo but sulfanilamide in a base of cod-liver oil, oleic acid, beeswax, triethanolamine, and water was successful. Treatment of impetigo over a period of six to eight days showed complete healing in milder cases of impetigo and all traces of scars disappeared in five days. A case of bullous impetigo also responded to treatment.

Pavone (30) found that an ointment composed of sulfathiazole, chlorobutanol, urea, and water in a vanishing cream base was satisfactory when applied to burns for postoperative dressings and necrotic areas. It also proved of value in the treatment of trichomonas vaginalis vaginitis when applied with a tampon.

For second degree burns involving not more than 20 per cent of the burned surface Pickrell (31) sprayed the burned area with a a sulfadiazine triethanolamine solution and applied a sulfadiazine ointment containing stearic acid, anhydrous lanolin, white wax, cetyl alcohol, petrolatum, triethanolamine, and carbitol. After twenty-four to forty-eight hours a culture was taken from the burned area, and the treatment was repeated if necessary. The results of treatment of 115 burned patients were gratifying.

Bigger and Hodgson (32) observed that a Lassar's Paste containing sulfonamides helped to prevent and to cure secondary infection. In treating impetigo contagiosa, ten out of eleven cases were cured with Lassar's Paste containing sulfapyridine, thirteen cases out of fourteen were cured with Lassar's Paste containing sulfanilamide, while only fourteen cases out of nineteen were cured with Lassar's Paste without sulfa drug.

Weiner (33) found that the seldom-occurring cutaneous hypersensitivity of topical application of sulfathiazole ointments was not caused by the ingredients of two commercial sulfathiazole ointment bases but by the drug itself.

Kern, *et al.* (34) used several different types of treatment in 360 cases of burns and obtained the best clinical results with 3 per cent sulfathiazole in a vanishing cream water-soluble base.

In the study of the etiology and treatment of impetigo, Sheehan and Fergusson (35) used a sulfanilamide cream containing 30 per cent sulfanilamide in oleic acid and cod-liver oil base, a sulfanilamide ointment containing 30 per cent sulfanilamide in an oleic acid, lanolin base and sulfathiazole in Mumford's base (5). Ten per cent sulfathiazole in Mumford's base proved to be the best since only 3.45 per cent of the cases were not healed by the ninth day, 30 per cent sulfanilamide ointment was second best since 5.64 per cent of the cases were not healed by the ninth day while 30 per cent sulfanilamide cream was least effective since 14.8 per cent of the cases were not healed by the ninth day.

In a study of local therapy of catarrhal conjunctivitis with sulfonamide compounds, Thygeson and Braley (36) found that emulsions and jellies gave more rapid release of sulfa drugs than ointments but were more irritating to the conjunctiva. Continuous

medication of the conjunctiva with the least irritation was obtained when a hydrous wool fat petrolatum base was used as a vehicle. Along the margins of the lids, however, where the preparation cannot be washed away by tears, jellies and emulsions were considered to be more suitable than ointments, since they were miscible with the exudation and allowed more intimate contact of the drug.

Leopold and Scheie (37) compared the intraocular penetration of an aqueous suspension of microcrystalline sulfathiazole with microcrystalline sulfathiazole ointment by introducing them in cats' eyes and analyzing specimens of the aqueous fluid at definite intervals. Subconjunctival administration of microcrystalline sulfathiazole did not excel the level of sulfathiazole obtained from the ointment. The addition of a detergent increased the penetration into the aqueous fluid of the eye of both the suspension and the ointment. More penetration was observed from the suspension than from the ointment.

Cole (38) pointed out that the majority of investigators preferred the oil-in-water emulsions in which the sulfa drug was suspended or dissolved in the aqueous phase, allowing even distribution of contact between the drug and the site being treated. Since this results in a lower surface tension, the compound mixed readily with the exudate and was easily removed by washing. Sulfathiazole is relatively effective with staphylococci and streptococci infections. Although the sodium salts of the sulfa drugs have the advantage of being readily soluble, they have the disadvantage of being highly alkaline. The sulfathiazole ointment of Pillsbury, Vammock, Livingood, and Nichols (12) with an oil-in-water base containing sodium lauryl sulfate, stearyl alcohol, cetyl alcohol, spermaceti, glycerin, and water was found to give good results.

Chamings (39) pointed out that the *p*H of the skin varies from 5 to 6 at different parts of the body. If absorption is desired oil-soluble medicaments are indicated in animal-fat bases. Insoluble constituents would appear to be relatively useless. If satisfactory medication of broken skin is desired an oil-in-water type of product may be ideal. In the investigation of the efficacy of sodium sulfathiazole in various bases, it was shown that paraffin or water-in-oil bases were relatively useless as compared with an oil-in-water emulsion. The properties of an ideal ointment base are (*a*) absence of irritant effect and absence of tendency to defat or dehydrate the skin, (*b*) miscibility with serum, (*c*) absence of inhibitory action on the therapeutically active constituents, (*d*) spreadability and anesthetic properties, (*e*) suitable *p*H, (*f*) ease of removal and nonstaining properties. The best bases for absorption of active ingredients are cod-liver oil, lanolin, lard, and goose grease.

Waud and Ramsay (40) studied the diffusion of sulfonamides from various ointment bases into agar media. The results suggest that the practice of incorporating water-soluble drugs such as the sulfonamides in fatty bases or water-in-oil emulsions should be discontinued. Much better results should follow the use of the sulfonamides in water-soluble bases. The sodium salt of sulfathiazole was found to diffuse a little faster than sulfathiazole itself. Thin layers of sulfathiazole preparations were found to deliver sulfathiazole almost as fast as thick layers.

Berens, de Gara, and Loutfall'ah (41) investigated the effect of sulfonamide ointment on the healing of experimental wounds of rabbit cornea and concluded that the average healing time of the wounds of the cornea treated with an ointment containing 2.5 per cent sulfanilamide and 2.5 per cent sulfadiazine and with the ointment base alone was usually greater than the average healing time of the untreated control eyes. Healing of deep incised wounds produced with a keratome infected with *S. aureus haemolyticus* occurred in one of three rabbit eyes treated with sulfonamide ointment but not in three untreated control eyes. A notable inhibition of the bacterial growth was observed in cultures of material from 8 to 24 eyes that had received treatment with the sulfonamide ointment but not in cultures of material from the control eyes. Local use of the ointment containing sulfonamide compounds in 5 per cent concentration

did not accelerate the healing of superficial or deep incised wounds of the cornea of rabbits if sterile instruments were used to produce the injury. The ointment was of value in the treatment of wounds produced with instruments that were infected with *S. aureus haemolyticus*.

Evans and Hoover (42) tested several sulfonamide ointments in the treatment of 126 cases of burns and found that the greasy base ointments were preferable to the water-dispersible base ointments because dangerously high blood levels of sulfonamides were avoided. The grease base used was composed of equal parts of sterile hydrous wool fat and cold cream to which was added 6 per cent sterile sulfanilamide by weight. Sulfanilamide rather than sulfathiazole or sulfadiazine was used because sulfanilamide results in less severe unfavorable reaction than do the other two sulfonamides.

In studying the penetration of sulfanilamide into the skin from various ointment bases, Strakosch and Clark (43) found there is no correlation between the penetration of sulfonamides from water-in-oil or oil-in-water emulsion type bases.

Barstow and Whittet (44) stated that the use of urea in sulfonamide ointments has been recommended to promote rapid healing and to abolish odors while recent work shows that it increased bacteriostatic action of sulfonamides and counteracted the inhibitory effects of such substances as *p*-aminobenzoic acid on the sulfonamides.

Fisher, Accousti, and Thompson (45) studied the bacterial contamination in sulfonamide ointments. Since ointments prepared with a water-dispersible base consisting of diglycol stearate, light mineral oil, cetyl alcohol, anhydrous wool fat, white petrolatum, and water produced only zones of partial inhibition when *S. aureus* was used as test organism, the ointment may become contaminated with disease-producing organisms. The addition of small amounts of other chemicals, particularly peroxides or germicides, increased their self-sterilizing properties.

In 68 cases of infection of the cervix, vagina, and vulva, Parks (46) used a special greaseless ointment containing allantoin, sulfanilamide, and lactose which was buffered to a *p*H of 4.5 with lactic acid. Surface infections of the exocervic, vagina, and vulva, with the exception of vulvovaginal mycosis, responded to local application of the ointment. This preparation was not curative for such lesions as condyloma acuminatum, granuloma inguinale, and lymphogranuloma venereum but it offered a relatively rapid means of reducing nonspecific, secondary infections to a minimum. Inguinal buboes and gonorrheal infection in the remote recesses of the urethra, endocarvical tubes, and vulvovaginal glands were influenced by this form of local therapy only in so far as they were effected by eradication of surface infections of the cervix, vagina, and vulva.

Bellows and Guttmann (47) found that an immediate factor in determining the transfer of drug from vehicle to the surface through which absorption took place was interfacial tension between the vehicle or solution and the tissue surface. Lowering the interfacial tension increased the rate of absorption of the drug. The authors found that the use of wetting agents with sulfonamides increased the penetration of the sulfonamides on the conjunctiva. The greatest effect was obtained from Aerosol OT and OS in 1 per cent concentrations and from 5 per cent bile salts. The concentration of sulfanilamide and sulfathiazole produced by wetting agents was greater than the concentration produced by oral administration of the drugs. The increase in penetration caused by wetting agents should therefore aid materially in building up and maintaining an adequate concentration in the tissues.

Aldrich, Savina, and Walsh (48) found that a combination of 3.75 per cent sulfanilamide and 1.25 per cent sulfathiazole suspended in a special oil-in-water emulsion base containing a wetting agent provided a very effective product in the treatment of lacerations, abrasions, minor burns, infections of vagina, furuncles, carbuncles, impetigo, infected leg ulcers, epidermophytosis, avulsed wounds, and infected eczemas. Out of 846 cases treated with this product only 4 showed signs of possible sulfonamide reaction.

Kaufman and Litterer (49) tested calomel ointment containing sulfathiazole as a prophylactic against gonorrhea on 100 individuals for the purpose of determining its irritability on the urethral mucous membrane. No discomfort was experienced at the time of the injection and there were no cases of chemical urethritis. This ointment proved its efficiency as a prophylactic against gonorrhea but since a control group was not used as a standard it was not known whether or not the addition of sulfathiazole to calomel ointment influenced its effectiveness as a prophylactic.

For the treatment of burns at the Montreal General Hospital, Larochelle (50) applied an oil-in-water emulsion containing 5 per cent sulfathiazole, triethanolamine, beeswax, liquid paraffin, and water in the form of a compression bandage with good results.

Buchi and Schlumpf (51) compared oil-in-water and water-in-oil emulsion ointment bases for sulfonamides and found that oil-in-water ointment bases gave better bacteriostatic action than the water-in-oil ointment bases.

After five years of clinical and laboratory study with 5 per cent sulfathiazole ointment Cochran (52) drew the following conclusions: (*a*) Sulfonamides in an oily or greasy base were no more effective clinically than plain petrolatum; (*b*) oily bases liberated only a trace of sulfonamides into a watery saline solution or into blood plasma even after twenty-four hours contact in a test tube; (*c*) much larger quantities of sulfonamides were liberated from oil-in-water emulsions than from oily bases; (*d*) the presence of a wetting agent offered even more available sulfonamide.

Devine (53) used allantoin-sulfanilamide ointment with a water-miscible base in extraction sockets and oral surgery. He found it superior to sulfanilamide powder and sulfanilamide powder in an ointment vehicle containing 2 per cent allantoin. The use of the ointment which combined the valuable properties of allantoin with sulfanilamide in the water-miscible base was found to give the best results.

It has been pointed out (54) that a sterile 5 per cent sulfathiazole ointment can be prepared with a base of triethanolamine, white beeswax, heavy mineral oil, and distilled water by using sterilized materials. This ointment should not be sterilized by heating since breaking of the emulsion will take place.

Busse (55) disclosed that when sulfonamide ointments were spread over the skin in thin films, the drug was absorbed rapidly from all types of preparations but when a compact mass of ointment was embedded in the tissues, absorption of the drug was delayed if the base was oily. An oil-in-water emulsion delayed the absorption somewhat but not as much as the oily base.

Woodward and his co-workers (56) determined the blood levels of sulfathiazole taken two and five hours after application and in catheterized urine samples taken three and one-half, six and one-half, and twenty-four hours after applying the ointment to 150 sq. cm. of dorsal clipped intact skin of rabbits. Using a sufficient number of animals so that differences reported were statistically significant, the following observations were made: (*a*) Sulfathiazole was absorbed poorly from fat or oil bases, slightly from water-in-oil emulsions and aqueous jelly, moderately from oil-in-water emulsions, and best from bases with high glycol content, propylene glycol being superior; (*b*) a sodium sulfathiazole or a microcrystalline sulfathiazole oil-in-water emulsion ointment failed to give absorption comparable to the glycol bases; (*c*) the addition of certain surface-active agents, notably sodium lauryl sulfate or an aryl alkyl polyether alcohol, improved the penetration of the ointment while certain others did not. These substances increased irritation; (*d*) skin penetration or localization from 5 per cent sulfathiazole powder equaled that from 20 per cent sulfathiazole ointments; (*e*) inclusion of calomel or organic arsenicals did not effect skin penetration; (*f*) skin localization was in evidence with glycol bases and increased with time of contact of ointments; (*g*) covering the site of application increased penetration.

Waud (57) measured on wounds of rabbits the rate and depth of diffusion of sulfa-

thiazole from water-miscible and fatty bases. He found that the higher the percentage of drug in the base the greater was the concentration found in the tissues. The delivery of sulfathiazole out of water-soluble bases or oil-in-water emulsion bases took place readily. When sulfathiazole was incorporated in fatty or petrolatum bases and applied to an open wound, little of the drug reached the tissues. When suspended in liquid oil, the drug settled out and was deposited in almost the pure state on the surface of the wound or dressing.

Cronkite, Deaver, and Dunn (58) experimented with 6 per cent sulfanilamide ointment and found that wound healing was optimum at *p*H 7.3, the activity of the sulfonamide increased with an increase in the *p*H, and almost all bacteria-infecting skin surfaces except pyocyaneus grew best in acid media. They found that a 6 per cent sulfanilamide in a base of white wax, anhydrous lanolin, mineral oil, water, and borax was smooth, easy to spread, and adhered well. This ointment has been employed successfully in the treatment of burns, impetigo, and around carbuncles to protect the skin.

Gurd and Gerrie (59) found that a 5 per cent sulfathiazole oil-in-water emulsion was helpful in the early plastic surgery of deep burns.

Good results in the treatment of burns (60) were obtained by the application of a cream containing 1 per cent cetyl trimethyl ammonium bromide, 3 per cent sulfanilamide in a base of castor oil, beeswax, wool fat, cetyl alcohol, glycerin, and water. The advantage of this cream was that it might be applied without preliminary cleansing.

Darke (61) found that 5.5 per cent of his group of patients were sensitive to an ointment of 5 per cent sodium sulfathiazole in a base of 30 per cent anhydrous wool fat, 40 per cent white petrolatum, and 25 per cent water. The contact dermatitis in each case disappeared when the sulfathiazole ointment was no longer applied but healing was definitely slowed by the ointment.

Saralegui and Villanueva (62) applied a greaseless ointment containing 2 per cent allantoin, 7 per cent sulfanilamide, and 40 per cent cod-liver oil and noted that sulfanilamide has a selective inhibitory effect on the various types of bacteria. The allantoin sulfanilamide mixture stimulated phagocytosis and accelerated the elimination of substances which inhibit healing. Also, it prevented the harmful effect of sulfanilamide, stimulated cell proliferation, and aided in the formation of new tissue.

Keeney, Ajello, Broyles, and Lankford (63) tested the effectiveness of sulfathiazole ointments with triethanolamine propionate-propionic acid and triethanolamine undecylenate-undecylenic acid bases in the treatment of tinea pedis. When tested *in vitro* the triethanolamine propionate-propionic acid base was superior in its fungistatic effect to the triethanolamine undecylenate-undecylenic acid base.

In a study of the absorption of sulfonamides from burned surfaces Evans, Hoover, and James (64) found that absorption was limited when oil base ointments were employed. In contrast, when water-dispersible bases were used, toxic blood levels of the drug may occur. An oil base composed largely of a mixture of lanolin and petrolatum proved to be satisfactory.

Jenkins, Allen, Owens, Schafer, and Dragstedt (65) studied clinically the action of sulfathiazole in the Aquafor base and the oil-in-water emulsion base and found that it was necessary to add some type of surface-active agent to the ointment base in order to obtain appreciable diffusion of sulfathiazole from a petrolatum base. It was thought that a surface-active agent might permit the emulsification of petrolatum with water thereby allowing the sulfathiazole to be liberated from the aqueous phase of the emulsion. The authors found that the surface-active agent permitted the liberation of sulfathiazole from the petrolatum without emulsification. Certain surface-active agents expedited the diffusion of sulfathiazole from petrolatum base in the surrounding aqueous media. In the Aquafor base series there were some instances in which proprietary bases did not liberate sulfathiazole any better than petrolatum alone. The emulsion ointment preparation demonstrated better original diffusion and con-

tinual diffusion than the Aquafor base ointments.

Aldrich and DeKay (66) tested a number of ointment bases for their ability to act as carriers for the sulfa drugs. An ointment base containing 10 per cent glyceryl monostearate, 5 per cent liquid petrolatum, 20 per cent lanolin, and 60 per cent water was found to give the best results. The optimum *p*H

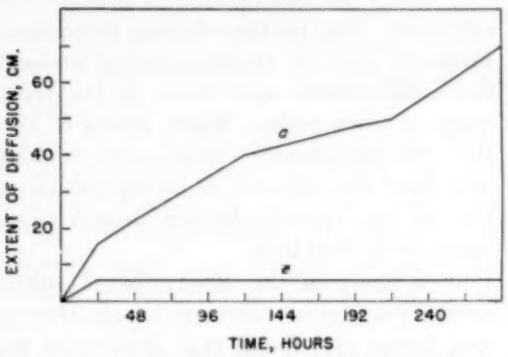

Fig. 1.—Oil-in-Water Base *vs.* Water-in-Oil Base.

a, Oil-in-Water Base: 5% Sulfathiazole U. S. P., 0.2% Cetylpyridinium Chloride, 10% Heavy Mineral Oil U. S. P., 35% White Petrolatum U. S. P., 0.8% Tragacanth U. S. P., 5% Propylene Glycol, 0.25% Chlorobutanol U. S. P., 43.75% Distilled Water.

e, Water-in-Oil Base: 5% Sulfathiazole U. S. P., 0.2% Cetylpyridinium Chloride, 1% Calgon Flakes, 10.5% Distilled Water, 83.3% White Petrolatum U. S. P.

was found to be around 9. The presence of about 50 per cent water was found to be necessary for optimum bacteriostatic activity. The authors found that surface tension reductants improved the penetration of the ointment as shown by the increase in the zone of inhibition.

The British National War Formulary (67) has recently recognized a sulfathiazole-sulfanilamide ointment containing glycerin, castor oil, Lanette wax SX, and water.

Recently Howard (68) has evaluated sulfonamide ointment bases by an *in vitro* method. A sample of the ointment to be tested was placed in a watch glass and then placed in a 6-ounce covered jar containing 100 cc. distilled water. The jar was incubated at 37.5°. At intervals of six, twelve, twenty-four, forty-eight, and ninety-six hours a 1-cc. sample of the water was withdrawn and assayed for sulfonamide content by the Bratton–Marshall method. Oil-in-water emulsions and aqueous jellies made with pectin or bentonite were found to liberate large quantities of sulfonamides.

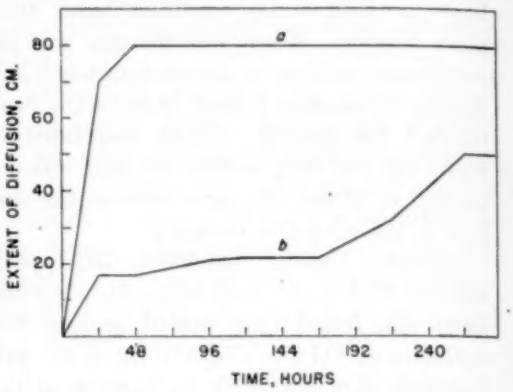

Fig. 2.—Water-Miscible Jelly *vs.* Water-in-Oil Ointment.

a, Water-Miscible Jelly Base: 0.1% Cetylpyridinium Chloride, 5% Tannic Acid U. S. P., 4% Benzyl Alcohol N. N. R., 5% Pectin, 0.05% Thiourea, 5% Sulfanilamide U. S. P., 5% Sulfathiazole U. S. P., 75.85% Distilled Water.

b, Water-in-Oil Ointment Base: 5% Sulfanilamide U. S. P., 5% Sulfanilamide U. S. P., 0.2% Cetylpyridinium Chloride, 1% Calgon Flakes, 78.3% White Petrolatum U. S. P., 10.5% Distilled Water.

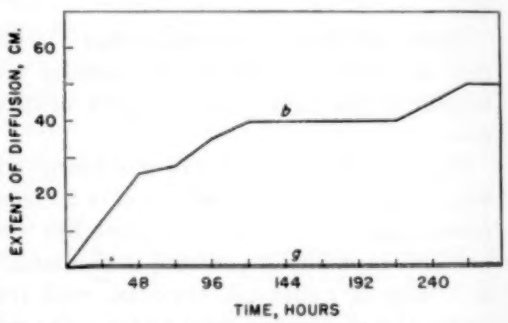

Fig. 3.—Water-in-Oil Bases with Different Amounts of Surface Active Agent.

b, With 10.6% Aerosol OT: 5% Sulfathiazole U. S. P., 11.6% White Wax, 10.6% Heavy Mineral Oil U. S. P., 37.2% White Petrolatum U. S. P., 10.6% Aerosol OT, 25% Distilled Water.

g, With 2.51% Aerosol OT: 11.02% White Wax, 10.07% Paraffin, 40.09% Heavy Mineral Oil U. S. P., 31.31% Distilled Water, 2.51% Aerosol OT, 5% Sulfathiazole U. S. P.

EXPERIMENTAL

The laboratory method used to evaluate the sulfonamide ointment and emulsion bases was the method of Waud and Ramsay (40) and it is quoted here directly from their work:

"A four per cent solution of agar in Ringer's solution was prepared and to this was added Ehrlich's solution which in contact with sulfonamides turns red. After being placed in test tubes and allowed to gel, a layer of the vehicle containing the sulfonamide was placed over the gel and the extent of diffusion measured at definite intervals."

The diffusion of sulfonamides from a number of ointments and jellies has been measured by this method and the results are shown graphically in Figs. 1–6. Each curve represents one measurement of the diffusion of that particular ointment or jelly. Ointments of opposite types and in some cases ointments of similar types containing the same amount of sulfonamide are considered in the same figure for the purpose of comparison.

Figure 1 shows the superior diffusion of sulfathiazole from the 5 per cent sulfathiazole oil-in-water emulsion base ointment *a* over the 5 per cent sulfathiazole water-in-oil emulsion base ointment *e*. These results are in agreeement with the results of Waud and Ramsay (40) and Howard (68). Figure 2 shows the superior diffusion of sulfathiazole and sulfanilamide from the water-miscible jelly *a* over a water-in-oil ointment *b* containing the same amount of sulfa drugs. In Fig. 3, two 5 per cent sulfathiazole water-in-oil ointments with different amounts of wetting agent Aerosol OT are compared as for diffusion of sulfathiazole. Sulfathiazole ointment *b* with 10.6 per cent Aerosol OT diffused more than sulfathiazole from ointment *g* with 2.5 per cent Aerosol OT. In Fig. 4 ointment *d* containing 10 per cent sulfanilamide and 10 per cent sulfathiazole in a water-miscible base diffused better than ointments *b* or *h* containing 10 per cent sulfanilamide and 10 per cent sulfathiazole in water-immiscible bases.

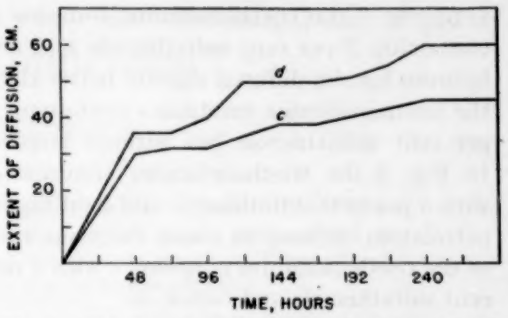

Fig. 5.—Triethanolamine Emulsions with and without Lanolin

d, With Lanolin: 26.66% Anhydrous Lanolin U. S. P., 4.99% Stearic Acid U. S. P., 1.76% Triethanolamine, 5% Sulfathiazole U. S. P., 61.59% Distilled Water.

c, Without Lanolin: 48.86% Linseed Oil, 5.55% Oleic Acid, 1.16% Triethanolamine, 5% Sulfathiazole U. S. P., 39.43% Distilled Water.

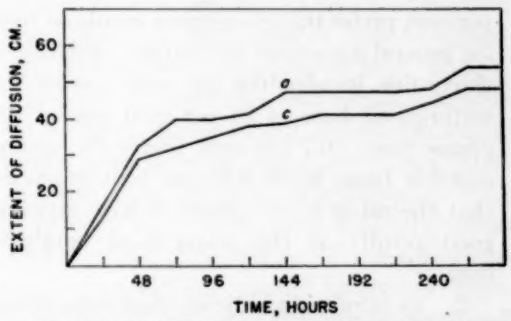

Fig. 6.—Triethanolamine Emulsions with Mineral Oil *vs.* Triethanolamine Emulsion with Vegetable Oil.

a, With Mineral Oil: 5% Sulfathiazole U. S. P., 2% Triethanolamine, 5% White Beeswax U. S. P., 64% Light Liquid Petrolatum U. S. P., 24% Distilled Water.

c, With Vegetable Oil: 5% Sulfathiazole U. S. P., 2% Triethanolamine, 5% White Beeswax, 64% Castor Oil U. S. P., 24% Distilled Water.

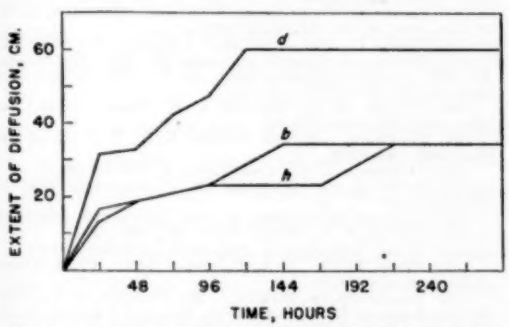

Fig. 4.—Water-Miscible Base *vs.* Water-Immiscible Bases.

d, Water-Miscible Base: 20% Carbowax 1500, 10% Heavy Mineral Oil U. S. P., 10% Sulfathiazole U. S. P., 10% Sulfanilamide U. S. P., 0.2% Cetylpyridinium Chloride, 49.8% White Petrolatum U. S. P.

b, Water-Immiscible Base: 0.2% Cetylpyridinium Chloride, 7% Distilled Water, 10% Heavy Mineral Oil U. S. P., 62.8% White Petrolatum U. S. P., 10% Sulfathiazole U. S. P., 10% Sulfanilamide U. S. P.

h, Water-Immiscible Base: 0.2% Cetylpyridinium Chloride, 5% Propylene Glycol, 7% Distilled Water, 10% Heavy Mineral Oil U. S. P., 57.8% White Petrolatum U. S. P., 10% Sulfathiazole U. S. P., 10% Sulfanilamide U. S. P.

In Fig. 5 the triethanolamine emulsion *d* containing 5 per cent sulfathiazole and anhydrous lanolin diffused slightly better than the triethanolamine emulsion *c* containing 5 per cent sulfathiazole but without lanolin. In Fig. 6 the triethanolamine emulsion *a* with 5 per cent sulfathiazole and light liquid petrolatum diffused at about the same rate as the triethanolamine emulsion *c* with 5 per cent sulfathiazole and castor oil.

SUMMARY AND CONCLUSIONS

1. Twenty-four of the investigators reviewed have compared the results of clinical investigation of sulfonamides suspended in oil-in-water base with sulfonamides suspended in water-in-oil base, grease base, or water-soluble base. Sixty-two and one-half per cent prefer the oil-in-water emulsion base for general use except for burns. Eight and thirty-five hundredths per cent prefer the water-in-oil base, 4.16 per cent prefer the grease base, 16.7 per cent prefer the water-miscible base, while 8.35 per cent maintain that the oil-in-water emulsion base gives as good results as the water-in-oil emulsion base.

2. *In vitro* tests indicate that sulfa drugs suspended in an oil-in-water emulsion type ointment base diffuse more readily than sulfa drugs suspended in the water-in-oil emulsion type ointment base.

3. *In vitro* tests indicate that diffusion increases with the solubility of the base.

4. The addition of a large amount of the wetting agent Aerosol OT increases the diffusion of sulfa drugs suspended in the water-in-oil emulsion type base.

5. The addition of lanolin to triethanolamine emulsions containing sulfathiazole increases slightly the diffusion of sulfathiazole.

REFERENCES

(1) Goodman, H., *Am. Profess. Pharmacist*, **8,** 355(1942).
(2) Anon., *ibid.*, **9,** 794(1943).
(3) Anon, *Pharm. J.*, **152,** 29(1944).
(4) Baker, W. B., "Useful Bases for Sulfa Drugs," Calco Technical Bulletin No. 686, Calco Chemical Division, American Cyanamid Co., February, 1944.
(5) Mumford, P. B., *Brit. J. Dermatol. Syphilis*, **50,** 540–43(1938).
(6) Robson, J. M., and Wallace, A. B., *Brit. Med. J.*, **1,** 469(1941).
(7) Anon., *J. Am. Med. Assoc.*, **116,** 356 (1941).
(8) Robinson, J. M., and Robinson, H. M., Jr., *Southern Med. J.*, **34,** 1093(1941).
(9) Keeney, E. L., Pembroke, R. H., Chatard, F. E., and Ziegler, J. M., *J. Am. Med. Assoc.*, **117,** 1415(1941).
(10) Keeney, E. L., *J. Am. Med. Assoc.*, **117,** 1911(1941).
(11) Veal, J. R., and Klepser, R. G., *Med. Ann. Dist. Columbia*, **10,** 61(1941).
(12) Pillsbury, D. H., Vammock, S. V., Livingood, and Nichols, A. C., *Am. J. Med. Sci.*, **202,** 808(1941).
(13) Guyton, J. S., *Am. J. Ophthalmol.*, **24,** 292–97 (1941).
(14) Thiessen, N. W., and Steinreich, O. S., *Military Surgeon*, **91,** 208(1942).
(15) Glicklich, E. A., *New Engl. J. Med.*, **226,** 981(1942).
(16) Miller, J. L., *Arch. Dermatol. Syphilis*, **46,** 379 (1942).
(17) Allen, J. G., Owens, F. H., Evans, B. H., and Dragstedt, L., *Arch. Surg.*, **44,** 819(1942).
(18) Kalz, F., and Prinz, M. V. H., *Can. Med. Assoc. J.*, **46,** 457(1942).
(19) Pillsbury, D. M., Schaffer, B., and Nichols, A. C., *J. Investigative Dermatol.*, **5,** 371–79(1942).
(20) Ackman, D., and Wilson, G., *Can. Med. Assoc. J.*, **46,** 209–14(1942).
(21) Ackman, D., and Wilson, G., *ibid.*, **47,** 1–7(1942).
(22) Gurd, F. B., Ackman, D., Gerrie, J. W., and Pritchard, J. E., *Ann. Surgery*, **116,** 641–57(1942).
(23) Strakosch, E. A., and Olsen, V. M., *Arch. Dermatol. Syphilis*, **46,** 44(1942).
(24) Winer, J. H., and Strakosch, E. A., *J. Am. Med. Assoc.*, **118,** 221(1942).
(25) Fuller, A. T., Hawking, F., and Partridge, N. W., *Quart. J. Pharm. Pharmacol.*, **15,** 136(1942).
(26) Elvin, N. C., *Arch. Ophthalmol.*, **27,** 373–74(1942).
(27) Hawking, F., *Lancet*, **2,** 507(1942).
(28) Wilcox, R., *Pharm. J.*, **148,** 217(1942).
(29) Wardropper, M. W., *Pharm. J.*, **149,** 16(1942).
(30) Pavone, J., *Am. Profess. Pharmacist*, **8,** 755(1942).
(31) Pickrell, K. L., *Bull. Johns Hopkins Hosp.*, **69,** 217–21 (1942).
(32) Bigger, J. W., and Hodgson, G. A., *Lancet*, **1,** 544 (1943).
(33) Weiner, A. L., *J. Am. Med. Assoc.*, **121,** 411(1943).
(34) Kern, R. A., Morris, R. F., Montgomery, H., Wigton, R. S., Brown, R. B., and Ferguson, L. K., *U. S. Naval Med. Bull.*, **41,** 1654–78(1943).
(35) Sheehan, H. L., and Fergusson, A. G., *Lancet*, **1,** 547(1943).
(36) Thygeson, P., and Braley, E. A., *Arch. Ophthalmol.*, **29,** 760–66(1943); cited by Bair, J. L., *Med. Clinics North America*, **28,** 790(1944).
(37) Leopold, I. H., and Scheie, H. G., *Arch. Ophthalmol.*, **29,** 811–17(1943).
(38) Cole, H. M., *J. Am. Med. Assoc.*, **123,** 411(1943).
(39) Chamings, A. R. G., *Pharm. J.*, **151,** 203(1943).
(40) Waud, R. A., and Ramsay, A., *Can. Med. Assoc. J.*, **48,** 121(1943).
(41) Berens, C., de Gara, P. F., and Loutfall'ah, M., *Arch. Ophthalmol.*, **30,** 631(1943).
(42) Evans, E. I., and Hoover, M. J., *Surg. Gynecol. Obstet.*, **77,** 367(1943).
(43) Strakosch, E. A., and Clark, W. G., *Am. J. Med. Sci.*, **206,** 518(1943).
(44) Barstow, E. W., and Whittet, T. D., *Charring Cross Hosp. Gaz.*, **43,** 71(1943).
(45) Fisher, C. V., Accousti, N. J., and Thompson, M. R., *J. Am. Med. Assoc.*, **122,** 855(1943).
(46) Parks, J., *Med. Ann. Dist. Columbia*, **12,** 175(1943).
(47) Bellows, J. G., and Guttmann, M., *Arch. Ophthalmol.*, **30,** 352–57(1943); cited by Blair, H. J., *Med. Clinics North America*, **28,** 791(1944).
(48) Aldrich, R. H., Savina, A. R., Walsh, R. A., *Ind. Medicine*, **13,** 693–97(1944).
(49) Kaufman, J. B., and Litterer, A. B., *U. S. Naval Med. Bull.*, **42,** 483(1944).
(50) Larochelle, J. L., *Laval Méd.*, **9,** 413–19(1944).
(51) Buchi, J., and Schlumpf, R., *Pharm. Acta Helv.*, **19,** 171–83(1944).
(52) Cochran, J. R., *Surg. Gynecol. Obstet.*, **79,** 326(1944).
(53) Devine, J. C., *Dental Digest*, **50,** 267(1944).
(54) Anon., *Am. Profess. Pharmacist*, **10,** 926(1944).
(55) Busse, L. W., *J. Am. Pharm. Assoc., Pract. Pharm. Edition*, **4,** 314(1943).
(56) Woodward, G., Wright, C. D., Evenson, C. L., Ofner, R. R., Kramer, D. S., Jenner, P. M., and Johnson, V. D., *Federation Proc.*, **3,** 87(1944).
(57) Waud, R. A., *Can. Med. Assoc. J.*, **51,** 229(1944).
(58) Cronkite, E. P., Deaver, J. M., and Dunn, W. W., *U. S. Naval Med. Bull.*, **42,** 1407(1944).
(59) Gurd, F. D., and Gerrie, J. W., *J. Am. Med. Assoc.*, **125,** 616(1944).
(60) Anon., *Chemist and Druggist*, **142,** 572(1944).
(61) Darke, R. A., *J. Am. Med. Assoc.*, **124,** 403(1944).
(62) Saralegui, A. F., and Villanueva, A. A., *Rev. asoc. méd. argentina*, **58,** 547(1944); through *J. Am. Med. Assoc.*, **127,** 125(1945).
(63) Keeney, E. L., Ajello, L., Broyles, E. N., and Lankford, E. V., *Bull. Johns Hopkins Hosp.*, **75,** 417(1944).
(64) Evans, E. I., Hoover, N. J., and James, G. W., *Surg. Gynecol. Obstet.*, **80,** 297(1945).
(65) Jenkins, H. P., Allen, J. G., Owens, F. M., Schafer, P. W., and Dragstedt, L. R., *Surg. Gynecol. Obstet.*, **80,** 85 (1945).
(66) Aldrich, D. F., and DeKay, H. G., THIS JOURNAL, **34,** 17(1945).
(67) Anon., *Pharm. J.*, **154,** 16(1945).
(68) Howard, R. W., *New Engl. J. Med.*, **232,** 698(1945).

The Status of Tyrothricin as an Antibiotic Agent for Topical Application*,†,‡

By JOHN HENDERSON

The various properties and applications of tyrothricin are reviewed. The information compiled concerning this antibiotic agent, which is not administered systemically, indicates that it may be an effective agent for the impregnation of adhesive compress dressings and other topical applications.

THE existence of that group of antibacterial and antifungal substances derived from living sources, known as antibiotics, long has been known. Pasteur and Joubert (1), as long ago as 1877, were aware that certain airborne organisms inhibited the growth of the anthrax bacillus, and they even suggested that this phenomenon of antibiosis might be useful in treating certain infections. The first serious attempt to apply this principle was made by Emmerich and Loew (2) in 1899, using pyocyanase, a substance derived from *Pseudomonas aeruginosa* (*Bacillus pyocyaneus*), which inhibited the growth of diphtheria, cholera, typhoid, and plague organisms. In spite of the pursuance of similar lines of investigation by many workers, no purified product of therapeutic usefulness was isolated until the preparation of tyrothricin in 1939 by Dubos (3–8). Although ten years previously, Fleming (9) of St. Mary's Hospital, London, had reported the discovery of penicillin, the actual isolation of this substance in a form suitable for clinical use was not reported until August, 1940, as the result of a cooperative investigation under the leadership of Florey (10).

Much attention has been directed toward the isolation of other antibiotic substances, the most important of which is streptomycin, announced in January, 1944, by Schatz, Bugie, and Waksman (11). This substance, derived from the soil organism *Actinomyces griseus*, has been found to be clinically useful in treating certain Gram-negative infections.

One feature which penicillin and streptomycin have in common is that they are antibiotics whose therapeutic effect is achieved principally by systemic administration, thus differentiating these substances from tyrothricin, which, for reasons to be discussed, is used only for topical application, and never parenterally or orally. This characteristic aroused our interest when it became apparent, on epidemiologic grounds, that the topical use of sulfonamides and possibly penicillin by large masses of our population might result in widespread sensitization and drug-fast strains of bacteria. Furthermore, Meleney (12) and others have thrown serious doubt on the effectiveness of the sulfonamides in controlling wound infections when used locally. Because tyrothricin appeared to offer promise as a therapeutic improvement over the sulfonamides and certain other antibacterial agents for topical application, and a means of avoiding the dangers possibly inherent in the indiscriminate use of the sulfonamides and penicillin, the desirability of utilizing this agent for purposes of topical antisepsis was investigated.

That tyrothricin, which is a mixture of two substances, gramidicin and tyrocidine, exerts an antibacterial action against a large number of organisms is well established, although no study defining the exact extent of the bacterial spectrum for tyrothricin has as yet been reported in the literature. Unfortunately, most of the published reports concern themselves with a limited group of organisms and none presents a comprehensive review of the problem. The data obtained by the different investigators are not always comparable since the medium used and the cultural conditions under which the tests were performed vary widely.

* Received Dec. 5, 1945, from Johnson & Johnson, New Brunswick, N. J.
† Read in part before the Scientific Section of the Proprietary Association of America, New York, N. Y., Dec. 5, 1945.
‡ The accumulation of much of the data reported herein with respect to the antibacterial and sensitizing properties of tyrothricin was made possible by a grant from the Johnson & Johnson Research Foundation.

In cooperation with Dubos, a systematic survey of the effect of gramicidin and tyrothricin on a large variety of microbial species under standardized conditions was undertaken (13).

The tests were carried out in a medium containing 1 per cent serum albumin. It is well known that most antiseptics are inhibited in their action against bacteria by proteins in general and by serum proteins in particular. We have considered, therefore, that an inhibition test carried out in a medium containing a large amount of serum albumin would resemble more closely conditions prevailing in exudates than would be the case in a medium free of proteins. Gramicidin is unaffected in its antibacterial action by any concentration of serum albumin; in fact, as observed by Dubos, its activity seems to be appreciably increased in this medium. On the contrary, tyrocidine, the other component of tyrothricin, is, like other antiseptics, largely inhibited by albumin.

Although this survey is far from complete, the results at the present time permit a few statements which are summarized in Table I. The data presented deal with gramicidin. The comparative values for tyrothricin would be 2 to 5 times larger. Tyrothricin contains approximately 20 to 25 per cent gramicidin, and some 60 per cent tyrocidine. Although the latter substance also exhibits marked antibacterial activity *in vitro*, most of this activity is lost in the presence of animal tissues; this is probably due to the fact, already mentioned, that tyrocidine is inhibited by serum proteins. Most of the *in vivo* activity of tyrothricin therefore is due to its gramicidin content.

Furthermore, the data presented in the table refer to bacteriostatic levels. Gramicidin does not kill the spores of aerobic or anaerobic sporulating bacilli, but only inhibits their multiplication. In the case of a few nonsporulating species (group D streptococci in particular) the effect of gramicidin is essentially bacteriostatic. In general, however, the bactericidal level is very close to the bacteriostatic level and this is particularly true in the case of the pathogenic species which we have tested.

An interesting comparison of the relative speed of tyrothricin and penicillin in killing a standard strain of *Staphylococcus aureus* was made by Crowe, Fisher, Ward, and Foley (14). A series of pour plates were made at varying intervals of time after inoculating with one drop of a 24-hour Staphylococcus culture, a 5-cc. broth tube containing tyrothricin in a concentration of 1:20,000. Those poured within two to three minutes after inoculation showed a marked reduction in the number of organisms, and the plate poured at the end of one hour was sterile. The penicillin plates, on the other hand, showed no decrease in the number of organisms after one hour. A sharp reduction, however, occurred between the third and sixth hours and the twenty-four hour plate was sterile.

TABLE I.—PARTIAL LIST OF ORGANISMS INHIBITED BY GRAMICIDIN

Organism	Concentration of Gramicidin Required to Inhibit Growth (γ/Cc. of Broth Containing 1% Serum Albumin)
Diplococcus pneumoniae	0.005– 0.02
Streptococcus hemolyticus (groups A, B, C)	0.001– 0.05
Streptococcus hemolyticus (group D) including *Enterococcus* and *Streptococcus faecalis*	0.05 – 0.2
Streptococcus anaerobius	0.01 – 0.02
Lactobacillus	0.05 – 0.5
Staphylococcus aureus	0.5 – 5.0
Corynebacterium diphtheriae and diphtheroid bacilli	0.05 – 0.5
Mycobacterium tuberculosis	0.02 – 0.05
Bacillus anthracis	5.0 –20.0
Saprophytic aerobic sporulating bacilli	0.1 – 1.0
Clostridium tetani and other anaerobic sporulating bacilli	0.02 – 0.5
Neisseria gonorrheae	0.5 –10.0
Neisseria meningitidis	0.5 –10.0
Gram-negative bacilli	No inhibition

The antibacterial effect of tyrothricin under clinical conditions has been extensively investigated. Rammelkamp (15) used tyrothricin in the treatment of 58 localized infections and demonstrated that when applied to chronic ulcers of the extremities, tyrothricin resulted in sterilization and healing if the local infection was caused by *Streptococcus hemolyticus*, *Staphylococcus aureus*, or *Streptococcus faecalis*. He also noted encouraging results upon application of

this substance to mastoid cavities following mastoidectomies. Staphylococcic empyema and pneumococcic empyema responded poorly, whereas in one patient with streptococcic empyema the response was dramatic.

Herrell (16) believes that the best results are obtained in cases of ulcers infected with *Streptococcus pyogenes*, and that the results against *Staphylococcus aureus* are not quite so satisfactory. In one series of 93 cases suffering from infected ulcers and wounds, reported from the Mayo Clinic, failures occurred in 17 (16). The response in more than half of the remaining cases could be considered good or excellent. Francis (17) reports a case of a sulfonamide-resistant infection with *Streptococcus pyogenes* which responded satisfactorily to local applications of tyrothricin, and Rankin (18) reported excellent results from the use of tyrothricin in five or six cases of chronic ulcer.

Kvale, Barker, and Herrell (19), as the result of an extensive study of ulcers of both ischemic and stasis origin, believe that tyrothricin is particularly valuable and were impressed by its low toxic effect on the extremely delicate tissues concerned.

The Russian investigators, Gause and Brazhnikova (20), reported their clinical results in a series of 573 cases with gramicidin S, a substance which is similar to tyrothricin but is less highly refined and less active. This series included infected gunshot wounds, empyema, and osteomyelitis. Application of gramicidin S led to rapid disappearance of bacteria from the wounds, and these workers thought that the substance was particularly effective in preparing these wounds for subsequent surgical treatment.

Tyrothricin has proved useful in the preparation of infected ulcers and wounds for skin grafting and frequently facilitates the eradication of stubborn bacterial infections, thereby hastening the possibility of early grafting (21).

The use of tyrothricin in the treatment of diseases of the nose and throat has been reported by Schoenbach, Enders, and Mueller (22), who observed some partial effect upon streptococcus carriers, but not complete sterilization. Lindsay and Judd (23) investigated in considerable detail the possible value of tyrothricin in the treatment of chronic infections of the upper part of the respiratory tract, and for elimination of pathogenic bacteria from the tonsils of carriers. It was not evident that the duration of the infection was shortened by the use of tyrothricin, nor did the substance appear to alleviate the symptoms. Likewise, there was little evidence that improvement following the use of tyrothricin for chronic infection of the sinuses was any more rapid than customarily follows conservative treatment.

On the other hand, Crowe and his associates (14) used tyrothricin locally in the treatment of acute otitis media, acute and chronic mastoiditis, and acute and chronic sinusitis, for over two years and felt that they obtained better results in many cases than with other forms of treatment.

Tyrothricin has also been used for the treatment of such miscellaneous conditions as keratoconjunctivitis, pneumococcal conjunctivitis (24), treatment of Gram-positive bladder infections by instillation, and certain cases of pyodermatitis (15). Piper (25) has found the material effective in treatment of coccal and certain mixed infections of the vagina, but we doubt that it is clinically effective against trichomiasis.

The remarkable effects of tyrothricin in the treatment of bovine mastitis have been applied on a very large scale in the field and should be mentioned, since this work, which has been presented in great detail by Little, Dubos, and Hotchkiss (26–29), was largely responsible for attracting attention to tyrothricin for use in human medical fields.

The optimal therapeutic concentration of tyrothricin, taking into consideration the local tissue toxicity and the maximal antibacterial effect, as originally demonstrated by Herrell and Heilman (21, 30–32), is in the neighborhood of 500 micrograms per cubic centimeter. This concentration is widely used today for therapeutic application, and was adopted as the basis for calculating the amount to be impregnated per unit area of gauze in the manufacture of adhesive compress dressings.

That gauze impregnated with tyrothricin

in a manner such that, as the gauze becomes wetted, the tyrothricin with which it is impregnated automatically forms a solution in the tissue fluids of approximately optimal concentration, exerts well-marked bacteriostatic and bactericidal effects, is readily demonstrable.

In vitro studies, utilizing gauze impregnated with tyrothricin in a concentration of 70 micrograms per unit area of gauze, were carried out. Five cubic centimeters of aerobic broth were measured into each of 16 small test tubes, a tyrothricin-impregnated pad being placed in each tube, and the entire system autoclaved at 250° F. for twenty minutes. The tubes then were cooled to room temperature and inoculated with various dilutions of an overnight broth culture of *Staphylococcus aureus*, strain 6538, FDA 209. Incubation was at 37.8° C. Utilizing this technique, it was found that in dilutions greater than 10^{-1}, *Staphylococcus aureus* failed to grow except in one instance where growth occurred at a dilution of 10^{-2}. All of the controls showed growth at the end of sixteen hours. In the case of a highly virulent hemolytic streptococcus (Corley strain) isolated from a case of erysipelas of the breast, no growth occurred even in a dilution of $10-^{1}$ after incubation for twenty hours at 37.8° C. Similar results were obtained when a *Corynebacterium pseudodiphtherium* was used as the test organism.

These studies demonstrate two important facts: first, that the manner in which tyrothricin is impregnated into the gauze pads facilitates its availability as a bactericidal agent, and secondly, that the bactericidal activity of the tyrothricin is not diminished by autoclaving. This latter fact is of practical importance since autoclaving is, of course, essential to the preparation of a sterile finished product.

Until recently it has been misleading to use the standard agar-plate techniques for the comparative evaulation of the antibacterial activity of tyrothricin, owing to the fact that tyrothricin fails to diffuse readily through the agar media used in these methods; thus falsely low results are obtained. However, Dubos (33) has overcome this objection by utilizing a modified medium containing serum albumin. With this technique it has been possible to obtain satisfactory evidence of the antibacterial activity of tyrothricin-impregnated gauze pads against selected organisms.

However interesting the *in vitro* studies may be, it is only logical to inquire into the activity of the preparation under controlled experimental conditions which simulate as closely as possible those of actual use. With this thought in mind, two sets of studies were carried out.

The first of these makes use of the implantation of a known amount of tyrothricin-impregnated gauze into the peritoneal cavity of a mouse. One layer of gauze containing tyrothricin in a concentration of approximately 7 micrograms per square centimeter is introduced in the peritoneal cavities of mice weighing 30 Gm. Control mice receive the same amount of untreated gauze. Three hours after the implantation of the gauze, the mice are infected with 100 to 10,000 M. L. D. of pneumococcus type I. The mice infected after the introduction of normal gauze died of pneumococcal infection; those which received the impregnated gauze survived the infection. It is apparent, therefore, that adequate quantities of the tyrothricin are released from the impregnated gauze *in vivo* to protect mice against overwhelming pneumococcal infections.

The second study was designed to simulate the conditions under which tyrothricin is most widely used, that is, as an antiseptic topical application, and makes use of a method to be published by Dubos. The abdomen of a guinea pig is shaved and wiped with acetone. The skin is then irritated with an emery board, and with a scalpel small surface incisions in form of a cross are made at 4 to 6 sites, care being taken to avoid bleeding. One-twentieth of a cubic centimeter of a culture of hemolytic streptococcus group C or *Staphylococcus aureus*, diluted to 10^{-1} or 10^{-2}, is delivered from a tuberculin syringe and spread over each incision to form a small rectangular area. Each site then is covered with gauze. After thirty minutes, using a stiff loop of ni-chrome wire (large diameter) previously filled with sterile distilled water, the infected surfaces are sampled in five or

six slow movements, and each loopful is streaked on a blood agar plate. Then 0.05 cc. of various dilutions of the drug (diluted in 20 per cent propylene glycol, or 20 per cent propylene glycol alone for control) is applied to certain of the infected sites, and covered with gauze pads moistened with 0.15 cc. of the drug or control solution. To other areas, adhesive compress bandages impregnated with tyrothricin are applied.[1] All pads are left in place for two hours. Then the pads are removed and each site is streaked (sterile distilled water used as before) on blood agar. The data obtained from a study of this type are summarized in Table II.

TABLE II

Culture	Initial Bacterial Count (Plate Count), Approx.[b]	Drug	Pad	Final Count[c]
Hemolytic Streptococcus, Group C—Guinea Pig Virulent				
10^{-2} (10,000,000/cc.)	300	Tyrothricin 1/2000	Plain	0
	300	Tyrothricin 1/5000	Plain	0
	300	Tyrothricin 1/10,000	Plain	0
	300	20% propylene glycol	Plain	200
	300	20% propylene glycol	Tyrothricin Band-Aid	30
10^{-1} (100,000,000/cc.)	TMTC[a]	Tyrothricin 1/2000	Plain	0
	TMTC	Tyrothricin 1/5000	Plain	0
	TMTC	Tyrothricin 1/10,000	Plain	200
	TMTC	20% propylene glycol	Plain	TMTC
	TMTC	20% propylene glycol	Tyrothricin Band-Aid	300
Staphyloccocus aureus				
10^{-2} (10,000,000+/cc.)	TMTC	Tyrothricin 1/2000	Plain	10
	TMTC	Tyrothricin 1/5000	Plain	30
	TMTC	20% propylene glycol	Plain	300
	TMTC	20% propylene glycol	Tyrothricin Band-Aid	90

[a] Too many to count.
[b] 30 Min.—Platinum loop on skin, then streak on blood agar.
[c] 2 Hr.—Remove pad. Platinum loop on skin, then streak on blood agar.

From these data, it is clear that even though contaminating concentrations, higher than reasonably could be expected under clinical conditions, were used,[2] complete sterilization was achieved even with a 1:10,000 dilution of tyrothricin; when only the impregnated gauze was used, there was a reduction to a clinically insignificant number of organisms. The results are relatively as striking when an even more fantastic concentration of 10^{-1} (100,000,000 organisms per cubic centimeter) was used. In the case of a contaminating concentration of 10^{-2} of *Staphylococcus aureus*, dramatic reduction of the bacterial count, but not absolute sterilization, was obtained. In every case, however, the infection was reduced to the point where it was readily cared for by the tissues' own biologic mechanisms.

Some question may be entertained concerning the development of tyrothricin-fast strains of bacteria as this agent comes to be used by large masses of the population. Such a phenomenon is, of course, theoretically possible, as bacterial-fastness may be developed under certain conditions against almost any agent. In the case of tyrothricin, it is unlikely that such a phenomenon could be of practical significance, however, since the development of fastness to tyrothricin by a chance organism contaminating a specific wound would be a matter of purely local concern in so far as the treatment of that wound was concerned for the reason that there would be little possibility of such a tyrothricin-fast organism being disseminated and becoming established as a tyrothricin-fast strain among the population. Even though a given organism did become tyrothricin-fast, and subsequently caused a systemic infection, the fact would be of little material significance since some agent other than tyrothricin would of necessity be used

[1] Band-Aid sterile adhesive bandages, with tyrothricin-impregnated pad, were used in these experiments.
[2] A dilution of 10^{-2} of these cultures is equivalent to about 10,000,000 organisms per cubic centimeter.

to treat the condition. For this reason, tyrothricin is unique in that the theoretical possibility of the development of bacterial-fastness to this compound is of little epidemiologic significance.

Many antibiotic agents have proved of no practical use because of toxicity, even though some have exhibited a high degree of antibacterial activity. It is therefore of importance to consider tyrothricin from the standpoint of possible toxic manifestations before reaching a final conclusion regarding this substance.

That tyrothricin possesses a remarkably low local tissue toxicity was first demonstrated by the tissue cultures studies of Herrell and Heilman (30–32).

Dubos and Hotchkiss (34), in studying the components of tyrothricin, reported that high concentrations of gramicidin did not affect the oxygen uptake of polymorphonuclear cells, whereas similar concentrations of tyrocidine caused cessation of cell respiration and disintegration of white cells. Subsequent studies by Heilman and Herrell (31, 32) showed that gramicidin, on the other hand, was more toxic than tyrocidine and that the cytotoxicity of tyrothricin was largely due to its content of gramicidin. It should be emphasized, however, that according to Herrell and Heilman (32), when the cytotoxicity of gramicidin or tyrocidine is compared with that of other germicides commonly used for local application, such as zephiran, merthiolate or phemerol, these components of tyrothricin will be found to be considerably less toxic under the same conditions.

The acute toxicity of tyrothricin and its fractions for animals has been reported by Robinson and Molitor (35). They found that the daily administration to dogs of 2 mg. of gramicidin or tyrothricin per kilogram of body weight produced death in from two to eight days but were not toxic on oral administration. Gramicidin does not appear to be inactivated in the stomach, but the presence in the intestines of large amounts of Gram-negative organisms causes inhibition. There is little evidence that any therapeutic effect may be obtained by oral administration, although under experimental conditions, with the adequate feeding of gramicidin, a definite effect on the Gram-positive flora can be recognized.

The most striking toxic manifestation of tyrothricin is its hemolytic effect upon the red corpuscles when the material is injected experimentally into animals by the intravenous route. This phenomenon constitutes the principal reason that tyrothricin cannot be used parenterally; however, it is of no moment when tyrothricin is used topically or in the treatment of body cavities not directly connected with the circulatory system, or in the treatment of open wounds. Even in the latter case, should active bleeding be present, no absorption of the tyrothricin would be expected, since whatever tyrothricin were present would be washed away by the flow of blood before any absorption into the blood stream could occur; in any event, the amounts of tyrothricin present would be too small to exert any untoward effect.

Of importance is the question of possible sensitization to this substance. In reviewing the literature pertaining to tyrothricin, one is impressed with the paucity of reports concerning untoward manifestations resulting from the topical application of this material, in therapeutic concentrations, either to the body integument or various physiologic or artificial cavities. In so far as we have been able to ascertain, no instance of tyrothricin sensitivity has been reported, although the compound now has been used in a wide variety of conditions and applied by a number of different methods and vehicles over a period of several years. It is interesting to note that during the last two years tyrothricin has been produced in ever-increasing quantities and is being dispensed widely for a variety of purposes.

Grolnick (36) has exhaustively studied the question of whether tyrothricin was capable of causing sensitization. In 171 subjects on whom a solution of tyrothricin containing 1, 2, 5 or 10 mg. per cc. had been applied by patch test for a two-day period, there was no evidence of primary irritation. Twenty-five subjects who had been treated by a seven-day application with a solution containing 10 mg. per cc. did not become sensitized to tyrothricin. Finally, sensitivity to

tyrothricin failed to develop in forty-one subjects who had been given repeated applications with adhesive compresses impregnated with tyrothricin, over a prolonged period. Keefer (37) found no positive reactors in 105 patch tests, and Cooke and Finkelstein (38) tested a total of 82 subjects without observing evidence of sensitization.

It may be concluded with reasonable safety, therefore, that although sensitivity is not uncommon in the case of the sulfonamides, penicillin, and certain surface antiseptics, tyrothricin has not produced sensitization of the skin under conditions of the experimental studies so far carried out, and if it occurs clinically, experience would seem to be sufficiently large to warrant the conclusion that it must be very rare. However, were sensitivity to occur occasionally, this probably could not be considered of such serious consequences as would pertain in the case of the sulfonamides or penicillin, since tyrothricin is never administered systemically and sensitization would, therefore, be merely a matter of local contact and of no permanent significance.

CONCLUSIONS

The antibiotic agents, penicillin, streptomycin and tyrothricin are of major therapeutic importance. Penicillin is one of the most potent and least toxic of the systemic chemotherapeutic agents, streptomycin is effective against several Gram-negative pathogenic organisms not sensitive to penicillin.

Tyrothricin is useful against a wide range of organisms when applied topically, and in this respect its use should be considered complementary to that of the systemic antibiotics. It possesses advantages, for topical use, over certain other antibacterial agents, including the sulfonamides and penicillin, because of its stability, a wide spectrum of antibacterial activity, maintenance of potency in presence of blood and serum, low tissue toxicity, and lack of sensitizing properties. For these reasons, we believe that tyrothricin constitutes an excellent agent for the impregnation of adhesive compress dressings and other topical purposes.

REFERENCES

(1) Pasteur and Joubert: Quoted by Chain, E., and Florey, H. W., *Brit. Med. Bull.*, **2**, No. 1, 5(1944).
(2) Emmerich and Loew: Quoted by Florey, H. W., *Nature*, **153**, 40(1944).
(3) Dubos, R. J., *Proc. Soc. Exptl. Biol. Med.*, **40**, 311 (1939).
(4) Dubos, R. J., *J. Exptl. Med.*, **70**, 1(1939).
(5) Dubos, R. J., and Cattaneo, C., *ibid.*, **70**, 249(1939).
(6) Hotchkiss, R. D., and Dubos, R. J., *J. Biol. Chem.*, **132**, 791(1940).
(7) *Ibid*, **136**, 803(1940).
(8) Dubos, R. J., *Bull. New York Acad. Med.*, **17**, 405 (1941).
(9) Fleming, Alexander, *Brit. Med. Bull.*, **2** No. 1,4(1944).
(10) Chain, E., Florey, H. W., Gardner, A. D., Heatley, N. G., Jennings, M. A., Orr-Ewing, J., and Sanders, A. G., *Lancet*, **2**, 226–28(1940).
(11) Schatz, A., Bugie, E., and Waksman, S. A., *Proc. Soc. Exptl. Biol. Med.*, **55**, 66(1944).
(12) Meleney, F. L., *Surg. Gynecol. Obstet.*, **80**, 263(1945).
(13) Dubos, R. J., Personal communication, August 7, 1945; to be published with additional data.
(14) Crowe, S. J., Fisher, A. M., Ward, A. T., Jr., Foley, N. K., *Ann. Otol. Rhinol., Laryngol.*, **52**, 541(1943).
(15) Rammelkamp, C. H., *War Med.*, **2**, 830(1942).
(16) Herrell, W. E., *Surg. Clin. North America*, pp. 1163–76 (Aug., 1943).
(17) Francis, A. E., *Lancet*, **1**, 408(1942).
(18) Rankin, L. M. *Am. J. Surg.*, n.s., **65**, 391(1944).
(19) Kvale, W. F., Barker, N. W., and Herrell, W. E., *Med. Clin. North America*, pp. 849–59(July, 1944).
(20) Gause, G. F., and Brazhnikova, M. G., *War Med.*, **6**, 180(1944).
(21) Herrell, W. E., "Penicillin and Other Antibiotic Agents," W. B. Saunders Co., Philadelphia, 1945.
(22) Schoenbach, E. B., Enders, J. F., and Mueller, J. H., *Science*, n.s., 217(Aug. 29, 1941).
(23) Lindsay, J. R., and Judd, D. K., *Trans. Am. Acad. Ophthalmol.*, **47**, 431(1943).
(24) Heath, Parran, *J. Am. Med. Assoc.*, **124**, 152(1944).
(25) Piper, M. C., Personal communication quoted by Herrell, W. E., "Penicillin and Other Antibiotic Agents," W. B. Saunders Co., Philadelphia, 1945.
(26) Little, R. B., Dubos, R. J., and Hotchkiss, R. D., *Proc. Soc. Exptl. Biol. Med.*, **44**, 444(1940).
(27) *Ibid.*, **45**, 462(1940).
(28) Little, R. B., Dubos, R. J., and Hotchkiss, R. D., *J. Am. Vet. Med. Assoc.*, **98**, 189(1941).
(29) Little, R. B., Dubos, R. J., Hotchkiss, R. D., Bean, C. W., and Miller, W. T., *Am. J. Vet. Research*, **2**, 305(1941).
(30) Herrell, W. E., and Heilman, D. H., *Am. J. Med. Sci.*, **205**, 157(1943).
(31) *Ibid*, **206**, 26(1943).
(32) *Op. cit.*, p. 221.
(33) Dubos, R. J., Personal Communication, May 15, 1945; to be published.,
(34) Dubos, R. J., and Hotchkiss, R. D., *Trans. Stud. Coll. Physicians*, Philadelphia, **10**, 11(Apr., 1942).
(35) Robinson, H. J., and Molitor, H., *J. Pharmacol.*, **74**, 75(1942).
(36) Grolnick, Max, Personal communication, July 9, 1945; to be published in *J. Allergy*.
(37) Keefer, Chester S., Personal communication, June 22 1945.
(38) Cooke, R. A., and Finkelstein, M., Personal communication, Oct. 24, 1945.

Studies on Indian Rhubarb. II. *Rheum emodi**

HEBER W. YOUNGKEN†

A study of rhizomes and roots of Indian Rhubarb from Indian and American-grown plants is reported.

IN A paper published in May, 1944 (1), the author described the commercial Indian Rhubarb which was being imported into this country during 1943 and contrasted that material and galenical preparations made therefrom with Chinese or Tibetan Rhubarb of the U. S. P. and its official galenicals. Since that time plants of *Rheum emodi* Wallich and of other Indian species of *Rheum* have been grown by the author in the Medicinal Plant Garden of the Massachusetts College of Pharmacy and botanically authenticated plant materials and drugs have been received from different parts of India. It is the purpose of this paper to report studies made upon *Rheum emodi* plants and the drug obtained therefrom, the latter being shown by this investigation to represent one of the sources of the commercial Indian Rhubarb recently imported into the United States, and known to represent a variety of Himalayan or Indian Rhubarb used in the indigenous medicine of India (2) and found in the Indian bazaars (10).

Materials.—The materials used in this investigation consisted of leaves, rhizomes, and roots of *Rheum emodi* collected in Kashmir, India, herbarium sheets of overground portions and rhizomes and roots of *R. emodi* collected in the Lloyd Botanical Garden at Darjeeling, and in a nursery at Kashmir, India, three lots of dried *R. emodi* roots and rhizomes obtained from Kashmir, Nepal, and Dera Afghanistan through the Curator of the Indian Museum at Calcutta, all of which materials were secured through the courtesy of the U. S. Department of Commerce and the American Consulate General at Calcutta, also a number of plants of *Rheum emodi* obtained from Dr. M. F. Babb of the Cheyenne Horticultural Field Station, Cheyenne, Wyo., and a specimen of commercial Indian Rhubarb gathered in the Punjab district of India. The *Rheum emodi* plants from Cheyenne were transplanted into the Medicinal Plant Garden of the Massachusetts College of Pharmacy in October, 1943, and observations made upon them by the writer in 1944 and 1945, the data obtained being compared with taxonomic descriptions of the authorities cited later in this paper and found to agree in the main with them. Herbarium specimens of the overground portions of these were later verified as *Rheum emodi* Wall. by Dr. R. T. Clausen of Cornell University, an authority on *Rheum*. The rhizomes and roots of all of these materials were studied organoleptically microscopically, by qualitative chemical tests, and under filtered ultraviolet light. Tests were made to determine the percentage of diluted alcohol-soluble extractive of the dried and powdered drug and some preliminary tests have been made on two of the lots to determine their action. (Fig. 1.)

History and Description of *Rheum emodi* Wallich.—In his Catalogue of 1828 (3) Nathaniel Wallich listed *Rheum emodi* and cited two localities in India in which it occurred. It was found by Wallich at Emodus, a mountainous district of Gossain Than (3) and about Kamoun by Robert Blenkworth (4). Later, in 1932, Wallich described this species of Indian Rhubarb in his Plantae Asiaticae Rariores (5) as follows:

"*Rheum emodi* Wallich; racemis axillaribus subsimplicibus saepius aggregatis, terminalibus paniculatis, fructiferis strictis, pedicellis subternis fructu maturo dimidio brevioribus, floribus magnis, sepalis late ovalibus obtusis, careopsidis magnae faciebus oblongo-ovatis basi subcordatis apice integris obtusissimus, rarius subemarginatis, foliis integris obsolete undulatis in nervis subtus margineque hirtellis, junioribus supra adpresse hispidulis, caule superne pedunculisque puberulis." (Translation: Racemes axillary, nearly simple, often aggregate, in terminal panicles; fruits compactly arranged; subtending pedicels half as long as mature

* Received Nov. 1, 1945, from the Department of Materia Medica, Massachusetts College of Pharmacy, Boston.
† Professor of Pharmacognosy and Biology, Massachusetts College of Pharmacy.

fruit; flowers large, sepals broadly oval and obtuse; modified achene large, oblong-ovate, its base subcordate, its apex entire and obtuse, rarely subemarginate; leaves entire, obsoletely undulate, the nerves beneath and margin hirtellous; the younger leaves appressed hispidulous; upper part of stem and peduncles puberulous.)

He gave its habitat in the Himalayas up to altitude of 16,000 ft., in Tartary to Ladak, in Mt. Choor, at Gossain Than, in Kamoon. Before Wallich's description of this species appeared, Nees von Esenbeck and Ebermaier (6) published a brief description of it in 1831 under the name of *Rheum emodium* Wallich, a name which is non-valid under the Rules of the International Botanical Congress. However, they called attentiop to important characters of the leaf omitted in Wallich's description including the very weakly hairy leaf-stalk and the rounded-cordate blade both sides of which are clothed with short hairs.

In 1836, Sir Wm. J. Hooker published a more complete description of *Rheum emodi* in Curtis's *Botanical Magazine* (4) as follows: "Stem 6 to 10 ft. high, much branched and sulcated, very thick below, gradually attenuated upwards into large panicles and these rough with minute warts or excrescences, the color yellow-green streaked with red-brown. Leaves very large, gradually smaller upwards, roundish cordate, entire, somewhat wavy. Petioles thick, angled and furrowed, rough, embracing the stem by the large, bifid, sheathing membranous stipules. Panicles terminal, very long, the branches erect, virgate, rough. Pedicels solitary or clustered, somewhat verticillate, short, spreading. Flowers very small, of a deep blood-red color. Perianth of 6 spreading, ovate, deep segments, 3 alternate ones smaller. Filaments subulate, monadelphous at the base. Germen short, triquetrous, often aborted. Fruit dark blood colored, shining, an achenium which is cordate, triangular, the angles sharply winged, covered at base with persistent perianth. Seed ovate-triquetrous."

In 1890, Sir. J. D. Hooker described this species in "Flora of British India" (7), adding the following data: "Leaves long-petioled, very large, orbicular or broadly ovate; panicle papillosely puberulous, flowers dark purple, 1/4 in. in diameter. Radical leaves often 2 ft. in diameter, papillose beneath, subscaberulous above; petiole 12–18 in., scaberulous. Fruit 1/2 in. long, purple, wings narrower than the disk."

Fig. 1.—*Rheum Emodi* Wallich. On left, Upper portion of flowering plant grown in Medicinal Plant Garden of the Massachusetts College of Pharmacy showing stem bearing bracts and panicles of small flowers. On right, a radical leaf of the same plant.

In 1918, Kirktikar and Basu (8) illustrated the radical leaves, inflorescence, flowers and fruit of this species and briefly described it, but added little to what Wallich and the Hookers had previously published on it, the length of the fruit being reported by them being 1/4 in. They gave the habitat as Subalpine and Alpine Himalaya, Nepal, Sikkim, and Simba.

In 1922, Bailey, in "Standard Cyclopedia of Horticulture" (9), reported the following additional data: "petioles semi-terete, somewhat concave above; pedicels jointed below the middle; foliage coppery colored."

The literature on the pharmacognosy of *Rheum emodi* appears to be scant and is somewhat indefinite owing to the uncertainty of the authenticity of the drug described and the common practice of using the term "Indian Rhubarb" as synonymous

with *Rheum emodi*. Dymock (10) lists it as one of the three sources of the bazaar Rhubarb of India, and Dutt (11) merely cites hearsay data that the large, twisted, cylindrical segments and fairly odorous ones of Indian Rhubarb belong to *Rheum emodi*. Holmström (12) reported *Rheum emodi* grown in the Botanical Garden of Bern contained chrysophanic acid, traces of emodin, rhaponticin, rheochrysin, dextrose, and phytosterin.

Chopra (13) gives the results of the analysis by Elborne for *Rheum emodi*, low cultivated and *R. emodi* high cultivated. Ray, Guha, Bose, and Mukerji (2) have reported analytical data on ten different samples of Indian Rhubarb and have given a brief description of the cross section of the rhizome of *Rheum emodi*, comparing it with those of *R. palmatum* and *R. officinale*. They found two varieties based on the character of the medullary rays. In one, called "var. A," the medullary rays were arranged in several parallel rows, 2 to 3 cells wide, while in "var. B" the rays were continuous or discontinuous and consisted of a single layer of cells running along the radius. The botanical origin of their materials, however, is not definite, since they use *Rheum emodi* Wall. as synonymous with Indian Rhubarb.

The purgative properties and uses of *Rheum emodi* have been reported by Royle (14), Twining (15), and Chopra (13). Royle states that "Hill Rhubarb (*Rheum emodi*) was tried with excellent success and represents an effecient substitute for the foreign Rhubarb." Twining has reported favorably on its use in chronic diarrhea when that disease is accompanied by enlarged spleen and also of its effectual use in small doses as a tonic and astringent in the treatment of intestinal profluvia. In the London Dispensatory, 10th edition (1844), p. 688, he compares it with Chinese Rhubarb, stating that they accord so very closely in their medicinal powers that any of them may be used with equal certainty of success. Ray, Guha, Bose, and Mukerji (2) report the purgative action of Indian Rhubarb on cats to be delayed though quite definite, that cats are less susceptible to the action of rhubarb than human beings. In a series of 12 experiments they obtained an approximate idea that Indian and Chinese rhubarbs were almost identical in their purgative efficiency.

EXPERIMENTAL

Cultivated Plants.—The *Rheum emodi* plants obtained from Cheyenne, Wyo., and planted in the College garden at Jamaica Plain, Mass., attained a height of 6 ft. during the summer of 1945. They agreed for the most part in their essential features with the descriptions of Wallich, the Hookers, and Bailey, the chief differences noted being the color of the flowers which in their expanded condition varied from white to white tinged with purple. This may be attributed to the common tendency of albinism in purple flowered plants. The coppery green color of the foliage of this species noted by L. H. Bailey (9) was very pronounced in these plants.

While agreeing in their dominant external features with descriptions and herbarium material received from India, the internal structure of the rhizomes and roots of several of these plants differed from that of the Indian-grown plants in showing more numerous simple starch grains, fewer compound starch grains, in the walls of their tracheae being nonlignified except in rare instances, and in the behavior of their freshly cut transverse surfaces when exposed to filtered ultraviolet light, there being noted a velvety brown fluorescence in the bark region and a grayish purple fluorescence in the wood. A few rhaponticin crystals were also noted in a test made on the powdered material of these by the U. S. P. X method for Rhapontic Rhubarb (16). These internal variations point to the possible hybrid origin of some of the *R. emodi* plants obtained from Wyoming.

Mounted herbarium sheets of radical leaves of *Rheum emodi* Wall. collected in a nursery at Kashmir, India, by Mahismer Nath in 1944 and forwarded to the writer were long petiolate, the lamina cordate, 5-nerved, the apex obtuse, the base subcordate to cordate, the margin slightly and irregularly undulate and hirtellous. Similar radical leaves occurred in the Jamaica Plain grown plants. (See Fig. 2.)

Description of Various Lots of *Rheum emodi* Roots.—1. *Rheum emodi* Wall. From Curator, Industrial Section, Indian Museum. Botanical Survey of India, Calcutta. No. 16707. Source: Kashmir. The roots were undecorticate, conical, externally dark brown, deeply and irregularly longitudinally wrinkled and annulate, the fracture short and irregular, internally brown with a radiate wood. The odor was indistinct, the taste bitter and astringent. The sample gave a velvety brown fluorescence under the Hanovia Lamp. No rhaponticin crystals were evident when the powdered root was tested by the U. S. P. X method (16). The test for Rhapontic Rhubarb containing rhaponticin on particles of the powdered root placed in a mixture of H_2SO_4 and solution of furfural and examined under

the microscope, according to the method of Schuroff and Plettner, modified by De Rose and Wirth (17), was negative.

Sections and scrapings of the root showed the following histological features: Borke formation as far as the outer phloem region; a radiate wood; medullary rays from 1 to 3 cells in width; tracheae in wood wedges arranged singly, in groups of from 2 to 5 and in short to elongated radiate groups, mostly reticulate, a few spiral, their walls lignified; starch grains single and 2- to 3-compound, the individual grains spheroidal, irregularly spheroidal, ovate, dome-shaped or angularly convex, the hilum circular, triangular, resembling the wings of a bird in flight to several cleft, excentric, the grains up to 25 μ in diameter and exhibiting distinct polarization crosses and a marked play of colors with the selenite plate; rosette aggregates of calcium oxalate up to 136.8 μ; reticulate tracheae up to 152 μ in diameter.

2. *Rheum emodi* Wall. From Lloyd Botanical Garden, Darjeeling, India. The specimen consisted of an undecorticated rhizome and root system, externally black to grayish black, purplish where abraded, irregularly longitudinally wrinkled, both rhizomes and roots exhibiting an irregular short fracture, the fractured surface of the root showing a dark cambium separating a yellowish brown bark from a radiate wood of similar color. The odor was fragrantly aromatic, the taste bitter and astringent. The fractured surface gave a velvety brown fluorescence under the Hanovia Lamp, and tests for Rhapontic Rhubarb and rhaponticin by the U. S. P. X method (16), and the H_2SO_4-furfural method as modified by De Rose and Wirth (17) were negative. Transverse and radial-longitudinal sections of the rhizome showed the following characteristics: Passing from periphery to center: The periderm possessed several layers of partly exfoliated, necrosed, cortical tissues, followed by a phellogen (secondary), a zone of necrosed cortical tissue and another broad phellogen, a relatively broad phloem devoid of sclerenchyma, a wavy cambium line, a broad xylem of numerous wood wedges separated by medullary rays 1 to 3 cells in width, and a large central pith of starch- and crystal-bearing parenchyma. The wood wedges consisted of starch- and crystal-bearing wood parenchyma in which were imbedded mostly reticulate and a few spiral tracheae. The tracheae occurred singly in groups of 2 to 5 or in elongated radial groups which were frequently interrupted. In the inner third of the xylem of most of the sections examined occurred large groups of wood fibers in some of which tracheae were imbedded. The tracheae were mostly lignified. The rosette crystals were up to 91 μ in diameter; the reticulate tracheae up to 95 μ in diameter. The starch grains were single and 2- to 3-compound, the individual grains similar in outline and markings to those of *Rheum emodi* No. 1 but up to 27 μ in diameter. Polarization crosses were distinct and a marked play of colors was evident with the selenite plate. The 2 and 3 compound grains of this and the No. 1 specimens showed one of the component grains smaller than the others.

3. *Rheum emodi* Wall. Specimens from Nepal through Indian Museum, Calcutta. This material consisted of segments of the root from 1 to 4.5 cm. in length and up to 5 cm. in thickness, partially to completely decorticated. The outer surface varied from dark brown to purplish brown and was purplish to light brown where the periderm had been completely removed and was irregularly longitudinally wrinkled. The inner surface was yellowish brown, the smoothed inner surface showing a yellowish brown bark up to 5 mm. in thickness, an irregularly sinuate, dark brown cambium, and a yellowish brown, radiate wood. Pieces of the rhizome showed, in addition, a yellowish brown central pith. Under the Hanovia Lamp it showed a velvety brown fluorescence and was devoid of rhaponticin.

Fig. 2.—*Rheum Emodi* Wallich. Radical leaf of plant grown in Lloyd Botanical Garden, Darjeeling, India which yielded one lot of rhizomes and roots described in this paper.

The powdered material was brownish yellow in color and showed the following microscopical characteristics: Starch grains simple and 2- to 3-compound, rarely up to 4 compound, the individual grains spheroidal, plano-convex, or angular-convex, one of the components of the compound grains being usually smaller than the others, the hilum excentric, triangular to irregularly cleft, the lamellae frequently distinct, the grains mostly up to 19 μ, occasionally up to 27 μ, rarely up to 32 μ in diameter, and show-

ing distinct polarization crosses and a marked play of colors when examined with the selenite plate; reticulate and spiral tracheae and wood fibers with lignified walls, the reticulate tracheae up to 144 μ in diameter; rosette aggregates of calcium oxalate up to 100 μ in diameter.

4. *Rheum emodi* Wall. From Dera Afghanistan through Indian Museum, Calcutta. Specimen consisted of segments of dried roots, undecorticated, up to 6.5 cm. in length and up to 7 cm. in diameter, externally dark brown to purplish brown, deeply and irregularly wrinkled and furrowed longitudinally, internally showing a dull brown to yellowish brown bark, a dull, irregularly wavy cambium line and a yellowish brown, radiate wood. The powdered material was yellowish brown in color, with a moderately fragrant odor and a bitter and astringent taste. Under filtered ultraviolet light it showed a velvety brown fluorescence and was devoid of rhaponticin when tested by the same methods as employed in tests on the preceding specimens.

Histologically, the Dera Afghanistan material differed from the other purportedly authentic *Rheum emodi* roots received from India in possessing longer and broader medullary ray cells, in the maximum range in width of the medullary rays (1- to 4-cells as contrasted with 1- to 3-cells in the others), in the maximum size of the starch grains, many of which were up to 38 μ in diameter while some compound grains attained a diameter of up to 54 μ, and in the greater number of starch grains which were distinctly lamellated. The reticulate tracheae and rosette aggregates were up to 152 μ in diameter. The predominating shape of the simple starch grains was spheroidal but a number of ovate and pyriform grains also were present. The hilum varied from centric or excentric and, while occasionally found as a circular dot, most frequently occurred as an irregularly linear or branched cleft. The starch grains showed distinct polarization crosses and a beautiful play of colors with the selenite plate when examined under a petrographic microscope.

Examination of a recent commercial sample of Indian Rhubarb, reported to have been shipped from the Punjab district of India, showed it to closely resemble the Dera Afghanistan Rhubarb in its macroscopic and microscopic structure and in giving negative tests for Rhapontic Rhubarb (16) and rhaponticin (17). Accordingly, a portion of the present market supply of Indian Rhubarb represents *Rheum emodi*.

5. *Rheum emodi* Wall. P. I. 121986. Grown in Medicinal Plant Garden of the Massachusetts College of Pharmacy from plants received from Cheyenne Horticultural Field Station, Cheyenne, Wyo., Sept., 1943, and verified as authentic by R. T. Clausen. Rhizome systems of four plants were gathered in July 1945, washed, cut into segments, some of which were scraped and strung on wire and dried over a smoky fire, studied and ground to a No. 60 powder, and the remainder preserved in 50% alcohol for sectioning.

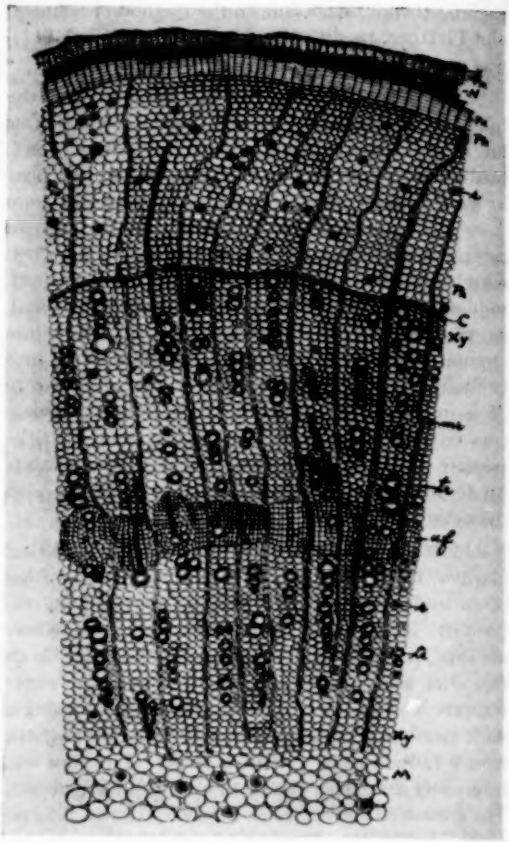

Fig. 3.—Transverse section of a representative portion of the rhizome of *Rheum Emodi* Wallich. K, cork; Pn, phellogen; N, necrosed bark tissue; Ph, phloem; C, cambium; Xy, xylem; r, rosette aggregate of calcium oxalate; mr, medullary ray; tr, trachea; wf, wood fibers; M, pith.

The material which had been dried over a smoky wood fire occurred in subcylindrical, plano-convex and irregular pieces, externally purplish brown, deeply longitudinally wrinkled and somewhat annulated. The fracture was short and horny, the fractured surface showing a grayish brown to yellowish brown bark, a dark, wavy cambium line, and a yellowish brown radiate wood. The odor was somewhat fragrant and slightly empyreumatic, the taste bitter and astringent. The powdered drug was yellowish brown. Under filtered ultraviolet light the bark portion exhibited a velvety brown and the wood a grayish purple fluorescence. The tests for Rhapontic Rhubarb by the U. S. P. X method and by the H_2SO_4-furfural test were positive, the fluid around the particles placed in the acid and furfural solution mixture varying from yellow to yellowish green to reddish purple.

Histology.—Transverse and longitudinal radial sections of the rhizome exhibited (*a*) a cork of suberous walled cells; (*b*) a phellogen of 12 or more layers of meristematic cells; (*c*) a narrow cortex of more or less tangentially elongated parenchyma cells with brownish walls and starch or crystal contents; (*d*)

a broad phloem separated into numerous phloem strands by phloem rays which are straight to slightly undulate in the inner region but which curve, often sharply in the outer region, and which are from 1 to 3 cells wide; the phloem strands consist of starch- and crystal-bearing parenchyma and sieve elements; (*e*) a wavy cambium line; (*f*) a xylem divided into a number of wood wedges by wood rays whose cells contain a yellow to orange substance taking a red color with alkaline solutions, the wood wedges consisting largely of starch- and crystal-bearing wood parenchyma in which are imbedded tracheae and wood fibers; and (*g*) a broad pith of starch- and crystal-bearing parenchyma.

Similarly cut sections of the root (14 mm. in diameter) exhibited (*a*) a nonlignified cork in process of exfoliation; (*b*) a phellogen of about 20 layers of cells whose radial walls appeared beaded; (*c*) a secondary cortex of several layers of collapsed tangentially elongated cortical parenchyma, the cells containing either starch grains or crystals of calcium oxalate; (*d*) a broad phloem of starch- and crystal-bearing parenchyma separated into numerous phloem strands by phloem rays whose cell contents were orange colored and were reddened by alkaline solution; the phloem rays are straight in the inner phloem but curve in their outward path; (*e*) a wavy cambium line; (*f*) a xylem whose wood wedges are separated by xylem rays, 1 to 3 cells in width, the contents of the ray cells being similar to those of the phloem rays. The center of the sections is occupied by primary xylem which consists largely of spiral and some reticulate tracheae. Sections through thicker regions of both rhizome and root showed a periderm of borke in which one or two phellogens were present. This region was followed by phloem whose rays extended to the secondary phellogen beneath the necrosed borke tissue.

The wood wedges of the root consisted largely of wood parenchyma in which were imbedded tracheae and wood fibers. The tracheae were arranged singly and in groups mostly of from 2 to 5, and were in many cases accompanied by wood fibers. In many of the sections studied there was a tendency for the wood fibers to be most numerous in the inner half or third of the secondary xylem.

In sections of two of the rhizomes studied, the inner extremities of the wood wedges were capped by wood fibers in some groups of which tracheae were imbedded. Stellate spots were absent in this as well as in other varieties of *Rheum emodi*. Most of the tracheae were nonlignified.

The starch grains were mostly simple, but 2- to 3-compound grains also occurred in which one of the component units was smaller than the others. The individual starch grains varied from spheroidal, dome-shaped to angular convex, the majority being spheroidal, and were up to 26.6 μ in diameter. The hilum varied in position from centric to excentric and in shape from spheroidal to a transverse or curved or branched cleft. Striations were distinct in reduced light. Under the polarizing microscope the starch grains showed distinct polarization crosses and a beautiful play of colors with the selenite plate. Rosette crystals of calcium oxalate were numerous and while most of them were less than 100 μ in diameter, a number occurred up to 136.8 μ in diameter, the largest found having a diameter of 152 μ.

Cross sections of freshly dug rhizomes and roots showed red to orange-red contents in the medullary ray cells, and the freshly cut cross surfaces of these organs showed red radiate streaks which, in the rhizome, extended outward from the white central disk of pith.

Anthraquinones.—Each of the five lots of *Rheum emodi* described in this paper was tested for emodin and chrysophanic acid by the U. S. P. XII method (18) and in every instance the result was positive for both of these constituents.

Diluted Alcohol-Soluble Extractive.—Determinations of the amount of diluted alcohol-soluble extractive were made on four lots of powdered rhizomes and roots of *Rheum emodi* earlier described in this paper in accordance with the U. S. P. XII method (19) with the following results. *Rheum emodi* (Kashmir) 35.28%; *Rheum emodi* (Nepal) 30.57%; *Rheum emodi* (Punjab) 39.97%; *Rheum emodi* (Jamaica Plain) 32.46%. All of them satisfied the U. S. P. standard for *Rheum* which is not less than 30%.

Pharmacologic Tests.—A series of preliminary tests to determine whether Indian Rhubarb from *Rheum emodi* possessed an emetic action was carried out on 12 persons to each of whom 20 gr. of powdered drug was administered. None of the subjects experienced emesis. Further work on the action of *Rheum emodi* is contemplated.

SUMMARY

1. The history and taxonomic descriptions of *Rheum emodi* Wallich are reviewed.

2. Descriptions are given of the physical characteristics and histology of five different lots of rhizomes and roots of *Rheum emodi*.

3. Locally cultivated plants of *Rheum emodi* obtained from Cheyenne, Wyo., are shown to agree in most of their external characteristics with the descriptions given by taxonomic authorities but the rhizomes and roots of three of these were found to differ from Indian-grown *Rheum emodi* in showing more numerous simple starch grains, fewer compound starch grains, in the walls of their tracheae being mostly nonlignified and in the freshly cut surfaces of their rhizomes and roots which, under filtered ultraviolet light, showed a velvety brown fluorescence in the bark and a grayish purple fluorescence in the wood. A few rhaponticin crystals were also

noted in a test made on some of the powdered drug by the U. S. P. X method for Rhaponticc Rhubarb. These internal variations point to their probable hybrid origin, although possessing dominant *Rheum emodi* characteristics.

4. Stellate spots are absent in the rhizomes of authentic *Rheum emodi*, and all of the rhizomes and roots of Indian-grown drug examined showed lignification of the walls of the tracheae.

5. Emodin and chrysophanic acid were found in both Indian- and American-grown *Rheum emodi*. Rhaponticin was absent in the *Rheum emodi* drug from India.

6. Determinations of diluted alcohol-soluble extractive on four lots of *Rheum emodi* drug showed the following percentages: *Rheum emodi* (Kashmir) 35.28%; *Rheum emodi* (Nepal) 30.57%; *Rheum emodi* (Punjab) 39.97%; *Rheum emodi* (Jamaica Plain) 32.46%.

7. A comparison of a recently imported lot of Indian Rhubarb from Punjab with authenticated *Rheum emodi* Root from Dera Afghanistan showed them to be of the same structure.

8. Preliminary tests made on twelve human subjects showed powdered Indian Rhubarb from *Rheum emodi* to be devoid of emetic action in 20-gr. doses. Further studies are contemplated on this variety of Indian Rhubarb.

REFERENCES

(1) Youngken, H. W., THIS JOURNAL, **33,** 145(1944).
(2) Ray, G. K., Guha, R. C., Bose, A. B., and Mukerji, B., *Indian J. Pharm.*, **6,** 55(1944).
(3) Wallich, N., Mss. Catalogue(1828), No. 1727.
(4) Hooker, Wm. J., *Curtis's Botanical Magazine*, London Botanical Magazine Warehouse, London, Eng., vol. 10 new ser. p. 3508.
(5) Wallich, N., "Plantae Asiaticæ Rariores," Treutte and Würtz and Richter, 1832, vol. 3, p. 65.
(6) Nees von Esenbeck, T. F. L., and Ebermaier, C. H., "Handbuch der medicinish-pharmaceutischen Botanik," Arnz & Co., Dusseldorf, 1831, vol. 2, p. 455.
(7) Hooker, J. D., "Flora of British India," L. Reeve & Co., London, 1890, vol. V, pp. 56, 57.
(8) Kirtikar, K. R., and Basu, B. D., "Indian Medicinal Plants," pub. by Sundhendra Nath Basu, Bahadurganj., Indian Press, Allahabad, Part II, 1918, p. 1081.
(9) Bailey, L. H., "The Standard Cyclopedia of Horticulture," The Macmillan Co., New York, 1922, vol. V, p. 2927.
(10) Dymock, Wm., Warden, G. J. H., and Hooper, D., "Pharmacolographia Indica," Kegan, Paul, Trench, Truner & Co., London, 1893, p. 155.
(11) Dutt, N. B., "Commercial Drugs of India," Thacker, Spink & Co., Calcutta and Simla, 1928, p. 204.
(12) Holmström, J. J., *Schweiz. Apoth. Ztg.*, **39,** 169, 183 (1921).
(13) Chopra, R. N., "Indigenous Drugs of India," The Art Press, Calcutta, 1933, pp. 235–36.
(14) Royle, J. F., *Transactions Medical and Physical Soc. of Calcutta*, **4,** 406–22(1829).
(15) Twining, Wm., "Clinical Illustrations of the More Important Diseases of Bengal," ed. 2 Baptist Mission Press, Calcutta, 1835, pp. 220–21.
(16) "United States Pharmacopœia" Tenth revision, J. B. Lippincott & Co., Philadelphia, 1926, p. 324.
(17) De Rose, A. F. and Wirth, E. H., *Pharm. Archiv.*, **15,** No. 6, 83(1944).
(18) "United States Pharmacopœia," Twelfth revision, Mack Printing Co., Easton, Pa., 1942, p. 393.
(19) *Ibid.*, p. 631.

WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

l-Methionine
Thiol histidine
3-Indole-β-acrylic acid
Ergothionine
α- or γ-Pyridone-3-sulfonic acid
Thymoform
Pyrene carbonic acid
Daphnetine
Isocrotonic acid
Allocinnamic acid
Hydroxy citric acid
5,11-Dihydroxy dibenzo (b,f,) (1,5)-dioxocin
2,2′-Dihydroxy-3,3′-di (1,2-benzopyran)
1,3-Dihydroxy indane
1,1′-Dihydroxy-3,3′-diphthalan
Montanic acid
Lignoceric acid
β-Hydroxy glutamic acid
Hydroxy aspartic acid
Lysolecithin (10 gm.)
Anabasin sulfate

Increase in Fatality Rate of E. typhosa for White Mice by Streptomycin*

By HENRY WELCH, C. W. PRICE and W. A. RANDALL

STREPTOMYCIN, an antibiotic metabolite of *Actinomyces griesus* was discovered by Waksman, *et al.* (1), in 1944. Shortly after this Jones (2), Robinson (3), and Heilman (4) reported that streptomycin, in contrast to penicillin, was highly active against many gram-negative organisms and some of the disease processes produced by them. Clinically streptomycin has been used with considerable success in the treatment of hemophilus influenzal meningitis, tularemia, and Gram-negative urinary tract infections, but questionable results have been obtained in other diseases which include tuberculosis, typhoid fever, and brucellosis. A review of the clinical data now available indicates certain unexplained results particularly in the latter two diseases. The etiological agents in these infections appear to be sensitive to streptomycin *in vitro* and, furthermore, successful treatment has been reported in some cases of both. Yet, even with relatively large doses, certain cases fail to respond to treatment even though blood levels of the drug are obtained in excess of those shown to be effective *in vitro*. Certain obvious answers suggest themselves such as invasion of the infecting organism into tissues which are not reached by appropriate concentrations of streptomycin; the interference of some material present in tissue or blood; or the rapid development of streptomycin fastness by the causative organism. Although these may provide an answer in some instances, in others they obviously do not offer a satisfactory explanation.

The purpose of this report is to describe a phenomenon which indicates that under certain conditions streptomycin actually increases the infectivity of the organism against which its antibiotic effect is directed.

Several isolated observations by various members of the laboratory personnel served to direct attention toward this somewhat puzzling phenomenon. In some preliminary work to determine bacteriostatic and bactericidal end points of streptothricin against *Staphylococcus aureus*, the nitrate-nitrite test described by Randall and Price (5) was used. It was noted that relatively high concentrations of streptothricin did not interfere with the ability of the organism to reduce nitrate to nitrite, while somewhat lower concentrations caused complete inhibition of nitrate reduction. However, lowering the concentration of the drug still further resulted in reduction of nitrates comparable to a control containing no streptothricin. Essentially the same results were obtained when streptomycin was substituted for streptothricin using two strains of *E. typhosa* as test organisms.

To determine if this same type of phenomenon could be demonstrated if the organisms were incubated in the presence of streptomycin, serial dilutions of streptomycin were made in broth and an equal quantity of a 1:100 dilution of a broth culture of *E. typhosa* added. After twenty-four hours 0.01 cc. of this mixture was transferred from each tube in the series in which there was no visible growth to a sterile Petri dish and melted agar added to form a pour plate. In all instances the last tube to show inhibition contained very few viable organisms while plates made from the one or two tubes preceding the last tube showing inhibition in the series, although containing larger quantities of streptomycin, contained large numbers of viable organisms. A similar result was obtained with three of four strains of *E. typhosa*. The details of these experiments will be reported elsewhere.

Another observation, which is being reported more fully separately, is that the addition of streptomycin to the diet of rats resulted in the elimination of coliform bacteria from their feces while the total count, although initially reduced, rapidly reached a

* Received May 7, 1946, from the Div. of Penicillin Control and Immunology, Food and Drug Administration, Federal Security Agency, Washington, D. C.

higher level than that observed in control animals even though the test rats were maintained on the streptomycin diet.

EXPERIMENTAL

To determine whether this phenomenon of apparent "stimulation" could be elicited *in vivo*, an experiment was designed in which *E. typhosa* (P. C. I. 412) was used as an infective agent in white mice and increasing concentrations of streptomycin were employed to protect, or to elicit, the "stimulation" phenomenon. The culture employed was a six-hour broth culture of *E. typhosa* and 0.2-cc. quantities, the LD_{80}, were used for intraperitoneal injections. Streptomycin was administered immediately following the injection of the organism by intraperitoneal administration of 0.5 ml. of a solution of streptomycin containing from 0.05 to 5.0 micrograms since preliminary *in vivo* determinations indicated that the "stimulatory" as well as the protective level was situated between these relatively narrow limits. It is of interest to note that these levels are the ones usually found in the blood of man at some time during treatment with this drug since the initial 10–20 γ/ml. level ordinarily found in the serum during the first one-half to one hour after injection rapidly falls to a level approaching zero within three to four hours. The experiment was repeated using 50 mice at each dosage level four times during a period of two months employing over 2000 mice. The results are set forth in Table I.

Inspection of Table I shows that one-half milliliter amounts of streptomycin solutions containing 0.05, 0.125, 0.25, 0.5, and 1.0 microgram rather than protecting an increasing number of mice as the concentration of streptomycin was increased as would be expected actually resulted in a marked increase in the percentage of deaths over the controls which did not receive streptomycin. These results are shown graphically in Fig. 1.

A subsequent experiment employing a 1:5 dilution of the LD_{80} dose gave results which appear even more significant. It will be noted (Table II) that the dose of organisms used caused the death of only 3.0% of the mice inoculated. Treatment of mice with 0.25, 0.5, and 1.0 microgram of streptomycin

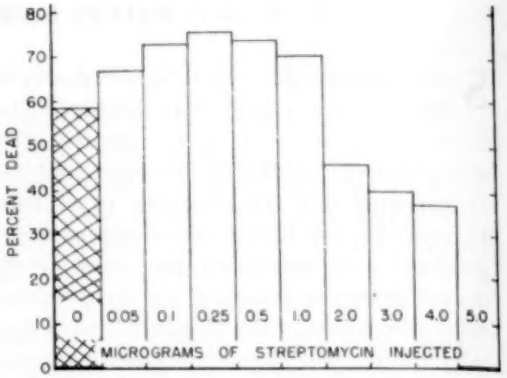

Fig. 1.—Effect of increasing concentrations of streptomycin when used to protect mice infected with *E. typhosa*.

TABLE I.—EFFECT OF INCREASING CONCENTRATIONS OF STREPTOMYCIN WHEN USED TO PROTECT MICE AGAINST *E. typhosa* (0.2 cc. = LD_{80})

Amt. of Streptomycin, γ	Mice Injected[a]	Number of Mice Killed				% Dead	% Survivals
		Expt. 1	Expt. 2	Expt. 3	Expt. 4		
0.0	200	31	32	28	26	58.5	41.5
0.05	100	...	...	30	37	67.0	33
0.125	100	...	...	38	35	73.0	27
0.25	150	...	36	38	39	68.5	24.6
0.5	200	39	37	37	35	74.0	26
1.0	200	39	36	35	33	71.5	29.5
1.5	200	23	23	24	22	46.0	54
2.0	200	23	23	21	13	40.0	60
2.5	200	22	16	7	9	27.0	73
3.0	100	...	15	...	6	10.5	89.5
4.0	50	...	7	...	...	14.0	86
5.0	40	0	0	...	....	0.0	100

[a] All mice received an injection of 0.2 cc. of a six-hour broth culture of *E. typhosa* just prior to the injection of the streptomycin.

TABLE II—EFFECT OF STREPTOMYCIN ON THE FATALITY RATE OF MICE INFECTED WITH A DILUTED CULTURE OF *E. typhosa*

Amount of Streptomycin Injected, γ	Number Mice Injected[a]	Total Number of Mice Killed	% Killed
0.0	200	6	3
0.25	200	40	20
0.5	200	48	24
1.0	200	57	28.5
1.5	200	8	4

[a] All mice received an injection of 0.2 cc. of a 1:5 dilution of a six-hour broth culture of *E. typhosa* just prior to the injection of the streptomycin.

injected immediately following the injection of the diluted culture of *E. typhosa* caused death of 20%, 24%, and 28.5% of the mice so treated and it was not until 1.5 micrograms of streptomycin were used that the percentage of animals killed approached that of the control (untreated) group. Statistical studies of the data obtained show that there is a highly significant increase in fatality rate due to quantities of streptomycin from 0.05 to 1.0 micrograms.

DISCUSSION

In vivo experiments employing *E. typhosa* and streptomycin by the intraperitoneal route in white mice indicate that at certain concentration levels of streptomycin, the organisms may actually undergo a "stimulation" resulting in a larger number of fatalities than would have occurred if the streptomycin had been withheld. The deduction that establishment of proper treatment schedules may result in clinical cures in conditions which now appear refractory to streptomycin is entirely logical; similarly, the deduction is logical that improper dosage schedules in conditions for which this drug is now being used may lead to undesirable results.

It is not possible with the facts now available to explain this phenomenon of apparent "stimulation" of a pathogen by streptomycin. However, it is possible to theorize on the basis of what is known concerning the effects of streptomycin on organisms susceptible to it. It is fairly well accepted that this antibiotic directs its inhibitory action on microorganisms by affecting some enzyme system concerned with growth and reproduction. It has been reported (6) that treponema pallidum and various other organisms (7) show marked increase in size in the presence of sublethal doses of penicillin. In our studies, huge, bizarre forms of *E. typhosa* have been noted in experiments concerned with increasing the resistance of this organism to streptomycin. It would appear that, as with penicillin, streptomycin stimulates growth of an organism but at the same time inhibits cell division perhaps by interference with the enzyme systems responsible for it. The possibility presents itself that in the so-called "stimulation" phenomenon reported here at certain concentration levels in the animal body, the stimulation in growth in size of the infecting organism, which is demonstrable microscopically, is carried over into the mechanism of cell division as well. Thus not only does the organism increase in size but in the number of divisions occurring in a given time interval. In a bacterial population such as was used in these experiments there is a variation in resistance of the individual cells to streptomycin. It could quite well be that it is this "resistant minority" in such a bacterial population that is responsible for the increased fatalities in mice observed with *E. typhosa*. These resistant organisms, which fail to succumb to concentrations of streptomycin which kill the weaker organisms, are stimulated to grow in cell size but because of their resistance, fission continues at the usual rate or possibly at an increased rate.

Another explanation along similar lines is possible. If it is true that streptomycin only acts on dividing cells it may be that cell division is retarded to such a degree by the antibiotic that fission fails to occur until after the antibiotic has disappeared from the animal body. However, regardless of the true explanation of the phenomenon observed in animals there is no adequate reason why such a "stimulation" effect cannot occur in man. If such is the case, the problem of proper dosage with this antibiotic is magnified tremendously. It should be noted that preliminary studies with penicillin indicate that a similar phenomenon is demonstrable at certain, though higher, concentration levels. These results are of particular interest in view of the recent and at present unexplained results reported by the Subcommittee on Venereal Diseases of the National Research Council and Public Health Service in both syphilis in man and in experimental syphilis in rabbits.

CONCLUSION

Streptomycin at certain concentration levels when injected intraperitoneally in mice infected with *E. typhosa* increases rather than decreases the fatality rate.

REFERENCES

(1) Schatz, A., Bugie, E., and Waksman, S. A., *Proc. Soc. Expil. Biol. Med.*, **55,** 66(1944).
(2) Jones, D., Metzger, H. J., Schatz, A., and Waksman, S. A., *Science*, **100,** 103(1944).
(3) Robinson, H. J., Smith, D., and Graissle, O., *Proc. Soc. Expil. Biol. Med.*, **57,** 226(1944).
(4) Heilman, F. R., *Proc. Staff Meetings, Mayo Clinic*, **19,** 553(1944).
(5) Randall, W. A., and Price, C. W., *J. Lab. Clin. Med.*, **29,** 312(1944).
(6) Frazier, Chester N., and Frieden, Edward H., *J. Am. Med. Assoc.*, **130,** 677 (1946).
(7) Gardner, A. D., *Nature*, **146,** 837–38(1940).

The Determination of Phenolphthalein in Mineral Oil Emulsions*

By MICHAEL FREDERICK† and ARNOLD KOFF

THE gravimetric methods for the determination of phenolphthalein (1–3) are too lengthy and cumbersome for routine control use. The red color produced in alkaline solution suggests a rapid method for the determination of phenolphthalein. The difficulties encountered in the colorimetric procedure are the stability of the emulsion and the fading of the red color of phenolphthalein in strongly alkaline aqueous solutions (4). The methods used in the gravimetric procedures to break the emulsion and to extract the phenolphthalein with ether-alcohol mixtures (1–3) failed to give satisfactory results with a colorimetric procedure. This was due to the residual solvent or other materials extracted from the emulsions which modified the color produced by phenolphthalein in aqueous alkaline solution. The difficulty was finely overcome by changing the solvent and eliminating the aqueous phase entirely.

EXPERIMENTAL

The most satisfactory means for breaking the emulsion and at the same time preparing the sample for analysis is to take up the emulsion directly in 95% alcohol.

It was found that in an alcoholic alkaline solution the red color of phenolphthalein was stable for a sufficiently long period to permit it being used in a colorimetric procedure. The optimum concentration of alkali was found to be 2 ml. of $N/2$ alcoholic KOH per 25 ml. of a solution of phenolphthalein in 95% alcohol. At this concentration the transmission was reproducible within 1% over a period of thirty minutes.

Figure 1 shows the absorption spectrum of the red color produced by phenolphthalein in an alcoholic alkaline solution. The maximum absorption occurs at 550 mμ.

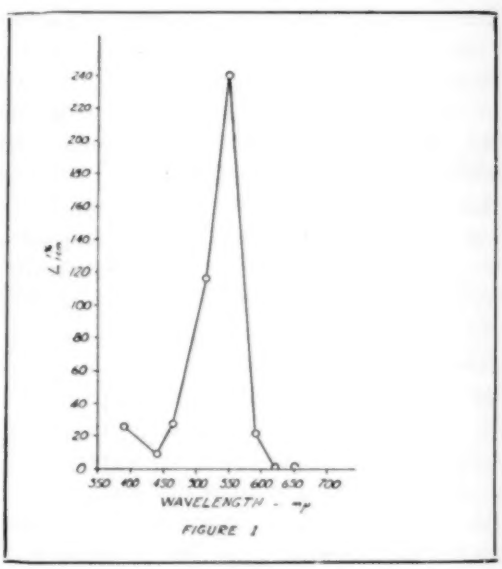

FIGURE 1

The term $L_{1\text{ cm.}}^{1\%}$ used to denote the extinction coefficient was introduced by Dann and Evelyn (5) and is analogous to $E_{1\text{ cm.}}^{1\%}$. The latter term is used with instruments which employ monochromatic light, whereas the term $L_{1\text{ cm.}}^{1\%}$ is used with photoelec-

* Received Dec. 4, 1945, from the Carroll Dunham Smith Pharmacal Company, New Brunswick, N. J.
† Present Address: Newark College of Engineering, Newark, N. J.

tric colorimeters using sharply filtered light of a band width of approximately 20–40 mμ.

The relationship between the light absorbed at 550 mμ and the concentration of phenolphthalein is shown in Fig. 2.

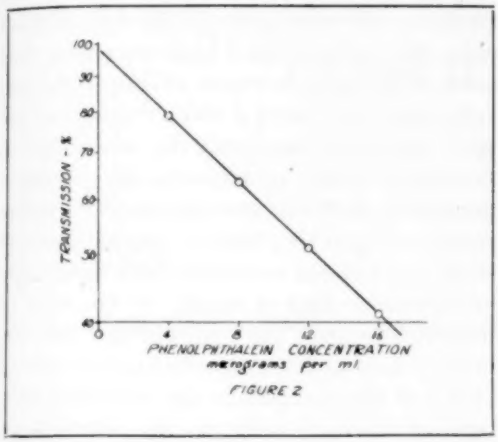

FIGURE 2

Assay Procedure

Preparation of Standard Phenolphthalein Solution.—Twenty milligrams of standard phenolphthalein is transferred to a 200-ml. volumetric flask and diluted to the mark with 95% alcohol.

Preparation of Sample.—A quantity of the emulsion representing about 50 mg. of phenolphthalein is transferred to a 200-ml. volumetric flask and diluted to the mark with 95% alcohol. The flask is shaken vigorously and the contents filtered. The clear filtrate is used in the color formation.

Color Formation.—To separate 25-ml. volumetric flasks are added 1-, 2-, 3-, and 4-ml. aliquots of the standard phenolphthalein solution and a 1-ml. aliquot of the assay solution. This is followed by 10 ml. 95% alcohol, 2 ml. $N/2$ alcoholic KOH, and then diluted to 25 ml. with 95% alcohol. The flasks are thoroughly shaken, and the per cent transmission of each solution against alcohol set at 100% is determined at 550 mμ on a Lumetron colorimeter model 402 EF, or other suitable instrument. The transmission values are plotted against the concentration of the standard dilutions on semilog paper. The concentration of the sample is interpolated from the graph.

The results of several assays of mineral oil emulsions for phenolphthalein are tabulated in Table I.

TABLE I

Sample	Colorimetric Assay, Gm./Oz.	Gravimetric Assay, (3), Gm./Oz.	Label Strength, Gm./Oz.
1	0.317	...	0.324
2	0.317	0.313	0.324
3	0.328	...	0.324
4	0.381	0.382	0.389

SUMMARY

A satisfactory method for the rapid determination of phenolphthalein in mineral oil emulsions is described.

Spectrophotometric data of the red color produced by phenolphthalein in alkaline alcoholic solution are presented, as well as the relationship of concentration of phenolphthalein to the per cent transmission of light at 550 mμ.

REFERENCES

(1) Bickford, C. F., and Schoetzow, R. E., THIS JOURNAL, **25**, 1128–29(1936).
(2) O'Keefe, H. F., *J. Assoc. Official Agr. Chem.*, **25**, 843 (1942).
(3) O'Keefe, H. F., *ibid.*, **26**, 311(1943).
(4) Hubacher, M. H., *J. Am. Chem. Soc.*, **65**, 2097–98 (1943).
(5) Dann, W. J., and Evelyn, K. A., *Biochem. J.*, **32**, 1008 (1938).

Book Review

Vitamins and Hormones, Volume III. Edited by ROBERT S. HARRIS and KENNETH V. THIMANN. Academic Press, Inc., New York, 1945. xv + 420 pp. 14.5 x 23 cm. Price, $6.50.

This now familiar review series has been continued and will remain an important tool to anyone in the vitamin or hormone fields.

The following reviews are included: "The Interrelation of Vitamins," by Thomas Moore; "The Synthesis of B Vitamins by Intestinal Bacteria," by V. A. Najjar and R. Barrett; "Sulfonamides and Vitamin Deficiencies," by F. S. Daft and W. H. Sebrell; "Manifestations of Prenatal Nutritional Deficiencies," by Josef Warkany; "Growth Factors in Microbiology—Some Wider Aspects of Nutritional Studies with Microorganisms," by B. C. J. G. Knight; "Possibilities in the Realm of Synthetic Estrogens," by E. C. Dodds; "Chemistry of Anti-Pernicious Anemia Substances of Liver," by Y. SubbaRow, A. B. Hastings, and Milton Elkin; "The Mechanism of Action and Metabolism of Gonadotropic Hormones in the Organism," by B. Zondek and F. Sulman; "The Role of Acetylcholine in the Mechanism of Nerve Activity," by David Nachmansohn.

This series of reviews is so broad in scope and covers so many papers (1442 references) that only a few highlights may be pointed out in such a brief review.

The reviews by Doctors Najjar and Barrett and Doctors Daft and Sebrell are closely related and show how the sulfonamides are serving as a tool to refocus attention on the important contribution of intestinal bacteria to nutrition. Daft and Sebrell state the problem very succinctly: "To the clinician they (the sulfonamides) have suggested that some of the so-called toxic effects of the sulfonamides may have a dietary origin or aspect and that they may be amenable to treatment, either prophylactically or therapeutically with specific nutrients. To the pharmacologist they have re-emphasized the importance of diet in studies of the toxicology and pharmacology of drugs. In the field of bacteriology they have reawakened interest in what we may from the teleological point of view call the function of the intestinal bacteria or, more broadly, in the influence of these bacteria for weal or woe on the host animal."

Doctor Warkany has provided a most dramatic review of the prenatal nutrition and its influence on offspring. The implications of Dr. Warkany's own work are highly important to human nutrition since he has been able to produce a high incidence of syndactylism, clubfoot, cleft palate, and other anomalies by dietary control of the mother. Further, he points out by statistical means the effect of dietary quality on the general state of health of newborn babies.

The chapter on "Anti-Pernicious Anemia Substances of Liver" points out that the amount of material (liver) needed by the patient has been reduced gradually from 400 Gm. to 1 mg.

Most of the other sections are of equal importance to the pharmaceutical research worker who will certainly wish to add this volume to his library—MELVIN W. GREEN.